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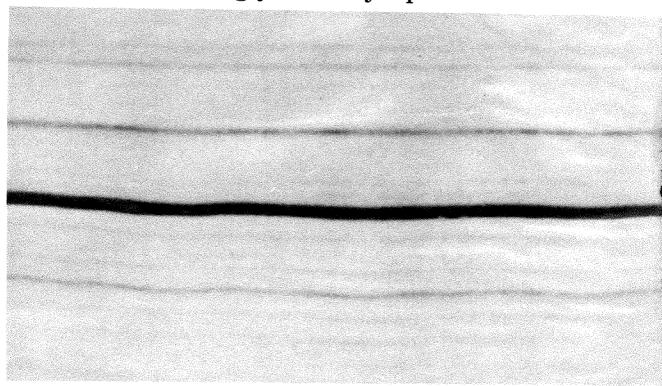
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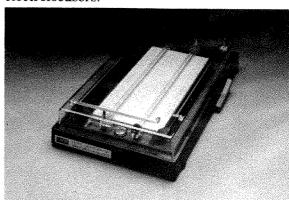
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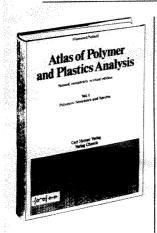
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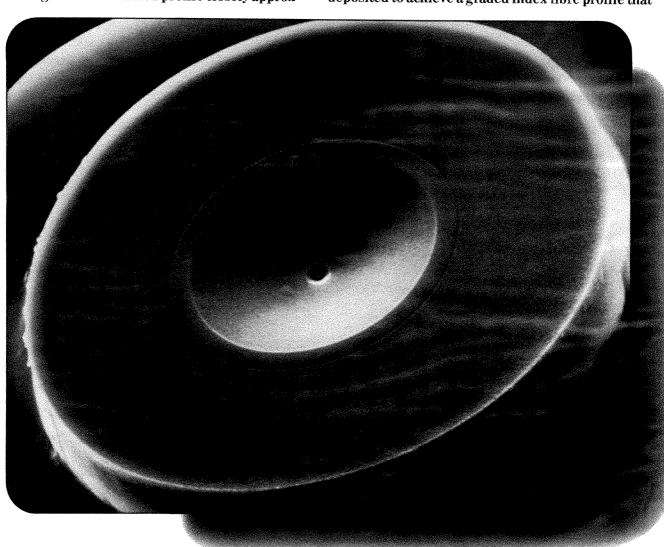
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An enlargement of the etched surface of an optical graded index fibre (50 $\mu$  core and 125 $\mu$  cladding diameter).



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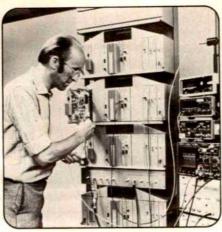
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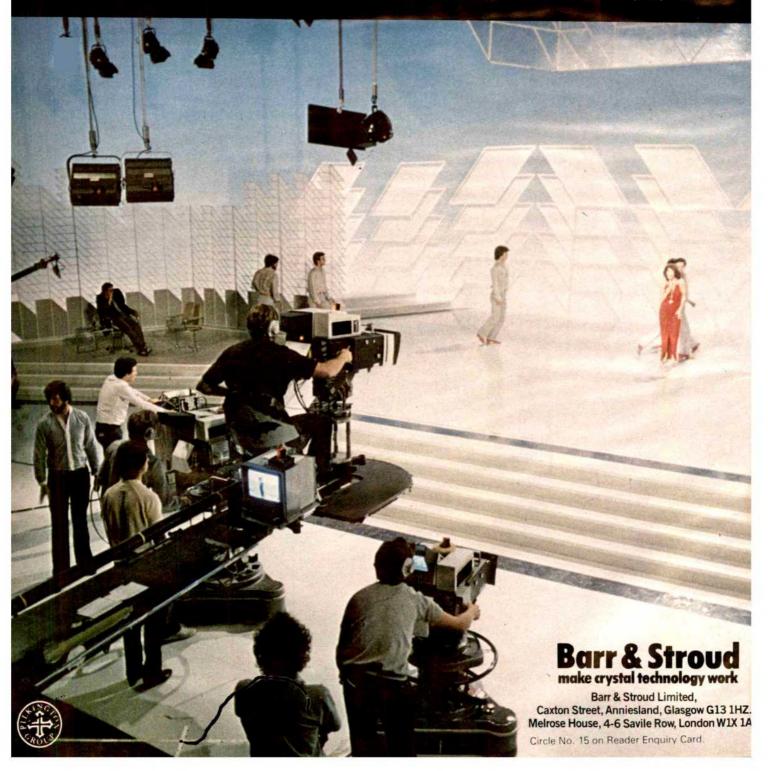
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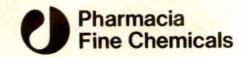
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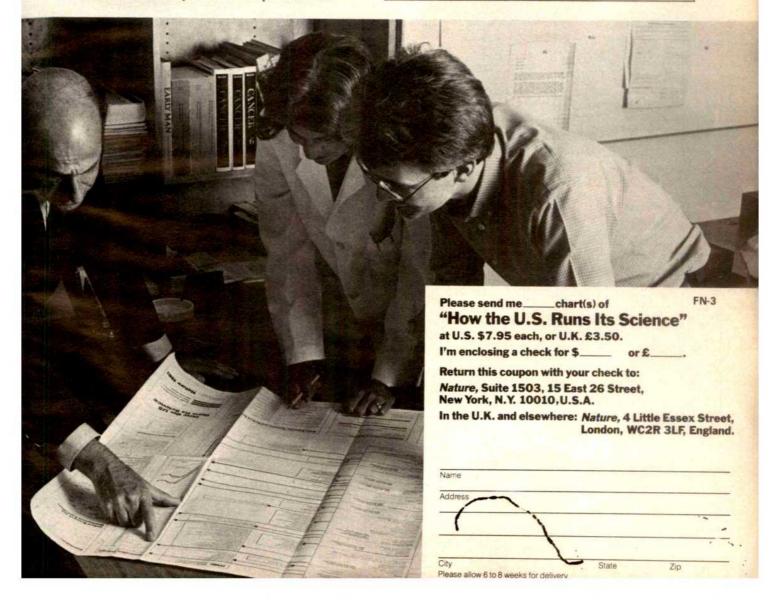
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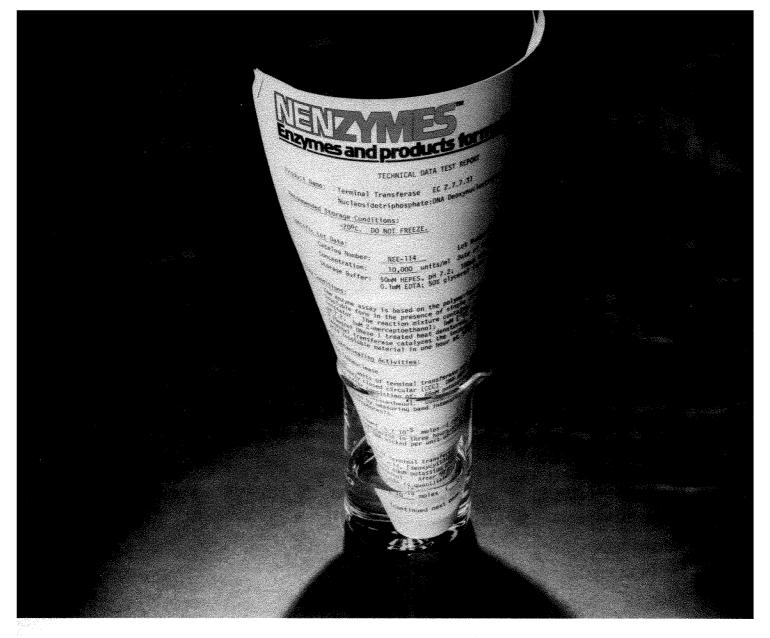
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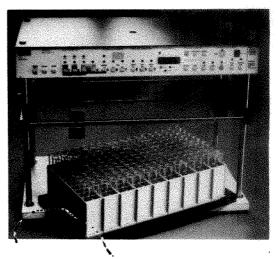
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5 November 1981

# TO YOUR TON

#### What Mr Blix should do

UN's atomic energy agency has been through a bad patch. Its new director should resurrect its original purpose.

The International Atomic Energy Agency in Vienna has plainly been scarred by the events of the past year, the Israeli raids on the Iraqi reactor at Tamuz among them. But for most of the year the agency has also been haunted by the unaccustomed difficulty of deciding who should be its new director general. Dr Sigvard Eklund, who until September had held the post for eight years, had not simplified the transition by making no secret of his willingness to serve for a third term: but the agency's governors were also perplexed in choosing between the two candidates on offer — Mr Hans Blix, deputy foreign minister of Sweden and Mr Domingo Siazon of the Philippines. The difficulty has been largely that of choosing between candidates from developed and developing countries. Exactly the same problem has more recently cropped up in the United Nations itself. The upshot of six months' heart-searching in Vienna is that Mr Blix has now taken up the post of director general. But he will probably be challenged again when his term expires four years from now. The danger is that the agency, hitherto almost as free from politics as the World Meteorological Organization, will fall foul of the squabbling to which most other United Nations organizations are prone. Mr Blix should therefore bend his energies to the insulation of the agency from the petty endemic politics of the United Nations system.

How is this to be accomplished? A sense of history will help. The Vienna agency is the product of President Eisenhower's programme in the 1950s that rejoiced in the slogan "Atoms for peace". Nobody doubted, then, that nuclear power stations would spring up apace in the territories of the nuclear powers, bringing prosperity in their wake: what more natural, even at the height of the Cold War, than that the United States and the Soviet Union should offer to share the benefits of their new technology with less fortunate governments? Even in the 1950s, however, people had forgotten that the agency they created had only its name in common with the much grander concept put forward in 1947, as part of the Baruch plan, of a United Nations agency that would literally be the sole owner and custodian of fissile material. The agency that Mr Blix has inherited is best known as the entity responsible for the administration of the safeguards required of non-nuclear power signatories of the Non-Proliferation Treaty: programmes of technical assistance continue, but on such a modest scale that they can make very little difference to the expectations of non-nuclear states that they will be able to decide for themselves even such simple questions as whether the much disputed benefits of nuclear power apply to them. The unaccustomed quarrelsomeness that has now emerged among the 111 member states at Vienna owes something to the belief that the agency should be doing more to spread such benefits of nuclear power as there are. The discontent is entirely justifiable.

The agency's most urgent need is for a realistic programme for helping developing countries to make sober appraisals of the utility of nuclear reactors. There is a limit to the extent to which they can be fobbed off with technical advice from international consultants on the place of nuclear technology in, say, agriculture. But where, the agency will ask, are the funds with which to support a more ambitious programme? For the time being, the unwillingness of the chief members of the agency to contribute more towards its budget is not its chief impediment.

At frequent intervals in the past two decades, the agency has published assessments of the place of nuclear reactors in the

economies of developing countries whose optimism has been matched only by their naivety — and which have nevertheless contributed to the present sense that the major nuclear powers are malevolently keeping nuclear power to themselves. Mr Blix should begin by commissioning a more realistic (and more technical) appraisal of the place of nuclear power in the smaller member states of the agency. He should then go further, for the assessment would almost certainly conclude that, within a decade, a great many smaller states will be able to make good use of the occasional well-sited and well-designed power reactor; he should offer a technical appraisal service for developing countries unable to decide between the claims of rival reactor salesmen.

Naturally, many of the founder members of the agency will object to such proposals. Appraisals that are too realistic may persuade some customers that the time has not yet come when they should buy. In the long run, however, that can only benefit the reputation of nuclear power and thus of its application. Others will protest that the encouragement of nuclear power can only assist the proliferation of nuclear weapons. The reply is twoedged. First, the involvement of the agency in the provision of civil nuclear power is likely to ensure that the safeguards procedures are effective. Second, the Non-Proliferation Treaty is a bargain in which non-nuclear powers agree to forsake nuclear weapons in return for a promise by the nuclear powers that they will assist with civil development. There is no way in which the nuclear powers can escape the commitment except by abrogating the treaty. Their best course is to help ensure that the safeguards procedures are continually improved.

To this end, Mr Blix could usefully consider whether the agency should soldier on as an amalgam of an international regulatory agency and a technical assistance organization. There is a strong case for reconstituting the safeguards organization as an independent quasi-judicial entity, perhaps a kind of permanent secretariat of the Non-Proliferation Treaty. Four years from now there will be a review of the working of the treaty. If safeguards are to be reorganized by then, it is not too soon to start. Fortunately, the wind is blowing in the right direction. The United States Administration is ready to loosen its restrictions on the export of reactors, but would find it convenient if the safeguards procedures were at the same time made more explicit. The agency's committee on the security of supply of nuclear materials, set up after the abortive conference in 1980 on the working of the Non-Proliferation Treaty, needs something tangible in which to sink its teeth. And with Mr Blix being a newcomer there is an ideal opportunity for change.

#### Congress versus cancer

The US Congress would legislate against cancer. It must be patient.

Part of the strength of democratically elected bodies, the United States Congress included, is that they are not necessarily bound by the decisions or the prejudices of their predecessors. That, however, is not a sufficient explanation of the way in which the present United States Congress, still less than a year old, seems bent on repudiating its predecessors' fondness for those agencies in the front line of what many congressmen used to call the "war

against cancer". Yet it is less than ten years since President Richard M. Nixon and Senator Edward M. Kennedy were vying with each other to devise still more generous programmes for cancer research. Over the years, the National Cancer Institute, a constituent of the National Institutes of Health, has been the centre of most of this attention, with the result that its operating budget now exceeds \$1,000 million a year. But something has gone wrong. Now hardly a week seems to pass without the director of the institute, Dr Vincent T. DeVita, appearing before one investigative committee or another to defend his institution against some fresh set of allegations. What can be the explanation? Has Congress fallen out of love with the war against cancer, or is there a more sinister explanation?

Part of the trouble is that Dr DeVita is new to the job — he took up his post only at the beginning of the year — and better known as a scientist than as a politician. Indeed, it seems that he has concentrated in the past few months on bringing a sense of direction to the research programme that he is paid to direct. This part of his job seems to have been done with such flair that when, earlier this year. Senator Hatch's committee sought to saddle him with responsibility for the earlier maladministration of research grants, a group of Dr DeVita's colleagues wrote expressing their admiration for their new director. Nobody would be surprised, however, if in the course of spending more than \$20 million a week on research, Dr DeVita has neglected to pay court as assiduously as he might to those who hold the purse strings in Congress. Or is it that Congress, like the National Institutes of Health, is waiting to see if the new director will turn out to be a political appointment, the Administration's representative within the institutes and not the institutes' means of interceding both with the Administration and with Congress? It goes without saying that a political appointment, a sharp break with precedent, could be disastrous for the National Institutes of Health, whose most urgent collective need is coherence and not further partition.

Dr DeVita's problems are nevertheless substantial. Accusations earlier in the year that some research grants had been used and administered in such a way that data of doubtful value had been gathered appear to have been substantiated. Neither then nor since, however, has Congress asked itself whether such incidents, acknowledged to be rare, can be avoided in such a gigantic programme for throwing money at the problem of cancer. Much of the same is true of last week's hearing in the House of Representatives, when Dr DeVita was required to deal with newspaper allegations that cancer patients treated with novel drugs within the framework of agreed experimental protocols had died as a result of the drugs rather than of the diseases the drugs might, with luck, have treated. How else, it might be asked, can novel drugs be tried in human beings? This week, Senator Paula Hawkins will return to the same charge; she has been asking, ever since she reached the Senate in January, why, a decade after the war against cancer was conceived of, people still die of all but a few forms of the disease. It is to be hoped that Dr DeVita and his colleagues will have the courage to tell Senator Hawkins the truth. The war against cancer was misconceived. The idea that such a problem might be made to go away by spending money was always destined to end in disillusion. From the start, it has been plain that while even successful methods of treatment lack a sufficient basis of understanding, they cannot be generalized. Yet, it must also be made clear, developments in the past few years promise substantial steps in the right direction, whatever that may

The practical consequences of such a confession on the part of the National Cancer Institute need not be as disastrous as they might seem. Congress may (separately from this week's proceedings) reduce the budget earmarked for the institute. It may have no choice. But the cancer institute would be more easily managed, and would be more effective, if it were forced to be more discriminating, at its very generous margin, in how its funds are spent. And the enterprise that it represents would be much less vulnerable than at present to the constant sniping that now afflicts it if both Congress and the United States public understood more clearly that they must be patient.

#### **Shame on New York**

Baseball has become a game of chance. How should it be reformed?

The city of New York has had a bad week. The leading article in last Thursday's New York Times was understandably entitled "The shape of New York's shame". Oddly, however, the newspaper was castigating the city council for a piece of flagrant but routine gerrymandering with the city's electoral boundaries, incurring as a result a reproof from the courts. But last Thursday morning, the shame hanging over the city was the humiliating defeat the previous evening of the city's most prosperous baseball team, the New York Yankees. In the process, the Yankees also lost the best-of-seven competition between North American baseball teams quaintly known as the "World Series". Last week's defeat was all the more galling because the Yankees were soundly beaten by the Los Angeles Dodgers, within living memory resident in Brooklyn and much resented for their defection. This gloom is yet another proof that something must be done to reform spectator sports in which the object is to hit a ball with some solid object, in baseball called a "bat"

At least to a first approximation, the interaction of a baseball and a bat is a soluble two-body problem. The bat in baseball is a simple object, cylindrically symmetrical about an axis, while the complication arising because some ball-throwers (called "pitchers") claim to be able to throw "curve-balls" is irrelevant to last week's disasters because both teams appear to have been incapable of hitting them. Moreover, since baseball officials have taken to measuring the speed with which individual baseballs are delivered, there is a wealth of empirical data on which a proper mechanics of baseball might be founded. Speeds of up to 40 metres per second are recorded.

The difficulty of baseball is, first, that of hitting the ball and, second, that of knowing where it will go when hit: good players appear to be able to hit one fair ball in three, but usually even the best players appear at a loss to know whether the ball will shoot high in the air (in which case it may be caught and they will be dismissed), vertically downwards into the ground or upwards and backwards, into the crowd. Only occasionally, perhaps half a dozen times in several hours, does the ball perform as every batter hopes, carrying over the boundary fence for a home run. Thus, it appears, baseball has become an elaborate game of chance. In this year's World Series, there seems to have been very little to choose between the batting performance of the two teams. Part of the reason why the Yankees lost is that they were not as skilled at catching and throwing the ball as were the Dodgers. It is now a matter of great importance to devise some way of ridding baseball of the luck on which it now depends, and which is a simple consequence of the circular cross-section of the baseball bat. Is there a place in baseball for the cricket bat?

Even New Yorkers will, however, agree that the most serious cause of the Yankees' defeat was the evil chance that attended one of the crucial decisions by the team's manager, Mr Robert Lemon. The issue is complicated by the requirements of this year's rules for the World Series that the pitcher, the man who throws the ball for his team, should also take his turn with the bat. At a point half-way through last Wednesday's game, Mr Lemon exercised his right to replace his successful pitcher due to bat with somebody reputed to be more skilled at hitting the ball, but who promptly failed to do so. Yet if the gamble had succeeded, the Yankees would have gained three runs and the Dodgers would have been too demoralized to sustain the struggle. What will happen now to Mr Lemon, who works for an owner so hottempered that he had to watch Wednesday's game with his hand in a cast after a fist-fight in a Los Angeles elevator, remains to be seen. Wisely, he has been keeping his own counsel. But is it not demeaning, and intolerable, that grown men's careers and the temper of a great city should be determined by the apparently random trajectories produced by trying to hit a baseball with a cylindrically symmetrical bat?

# Back to basics for high-energy physics

# US panel's ritual defence of accelerator

Washington

The high-energy physics community in the United States is lifting only the littlest of fingers in defence of the proton-antiproton accelerator ISABELLE at the Brookhaven National Laboratory, Long Island. At a day-long meeting on Sunday, the influential High Energy Physics Advisory Committee accepted a subcommitee's recommendation that the accelerator should be completed only if there were a prior assurance of adequate support for research at the two other accelerator laboratories at Stanford, California, and Fermi National Accelerator Laboratory (Fermilab), Illinois.

ISABELLE may thus turn out to be the most conspicuous casualty of the new financial restraint. The physical infrastructure of the machine, a tunnel 2.5 miles in circumference, is 95 per cent complete. But something like a further \$500 million would have to be spent on magnets, vacuum systems and radio-frequency accelerating cavities if the accelerator was to be finished by 1990.

If the project is abandoned, some 400 technical people at the Brookhaven Laboratory will be without jobs. There is even the gloomy possibility that, without its chief task, the survival of the laboratory itself will be in jeopardy.

The subcommittee report, the sole item

on Sunday's agenda, had been prepared in less than three months by a group under Dr George Trilling of the University of California, Berkeley. The panel will go on to produce a more measured statement in January of the benefits that may derive from particle accelerators based on novel principles such as those being developed at Stanford and Cornell universities. This

by the Department of Energy's need of an opinion from the high-energy physics community in advance of this week's crucial decisions within the Administration on how the latest 12 per cent budget cuts will be distributed.

week's interim report was made necessary

Given the conflicting interests of its members, the panel's chief conclusion is understandably delphic. It "strongly recommends" that ISABELLE should be completed, goes on to argue that the total cost of high-energy physics to the Department of Energy would then be \$440 million a year and remarks that if this support is not forthcoming, the ISABELLE project cannot be continued.

At present, high-energy physics is

operating on a budget of \$325 million a year, at which level the principal accelerators cannot be operated full time for lack of funds with which to pay the electricity bills.

There is every sign that high-energy physics has reached a turning point in the United States. Three distinguishable constituencies have emerged — the operators of the accelerators at Fermilab and Stanford, who wish to see their equipment fully used, the adherents of ISABELLE, who argue that it is an essential tool for the 1990s, and the general university users of accelerators, who need more immediate access to existing machines but who recognize that without ISABELLE they will have even fewer experimental opportunities ten years from now. The interim report spells out the conditions that must be satisfied before funds are spent on the completion of ISABELLE:

- Full utilization of existing accelerators.
- Completion of the Tevatron 1 accelerator upgrade for colliding 1,000 GeV protons and antiprotons based on existing Fermilab accelerators.
- A start on work on the Tevatron II

project, intended as the chief fixed-target accelerator for the 1980s.

- Development of superconducting radio-frequency cavities (at Cornell University).
- Development of novel accelerator designs.

Much of Sunday's meeting was occupied with the cost of this minimal programme, eventually fixed at \$395 million a year: if ISABELLE were to be completed, the total high-energy physics budget of \$440 million a year would include an average of \$80 million a year for the construction of the machine. If, however, ISABELLE is abandoned, the committee argued, it would be necessary to spend an extra \$35 million on research and development on new accelerator technology.

The committee's optimism that its "minimal" programme may be funded derives from the agreement in 1979 between the Department of Energy and the Office of Management and Budget that high-energy physics could count on a budget of \$300 million a year, adjusted for inflation. Several members argued that even with the completion of ISABELLE,

#### Top men resign in CNRS crisis

The two heads of the largest research agency in France — the Centre National de la Recherche Scientifique (CNRS) — fell last week in a self-delivered coup de grace.

On Wednesday, M. Jacques Ducuing, the director-general of CNRS, handed in his resignation to the minister for research and technology, M. Jean-Pierre Chevènement. Shortly after, the president of CNRS, Professor Charles Thibault — who worked in tandem with Ducuing — resigned also. He was followed by three of the six scientific members of the CNRS council, and the other three, including Louis Néel, a Nobel laureate representing the Académie de France, are likely to resign soon. (Néel, however, awaits the recommendation of the Académie.)

So what was the fuss? Chevenement was planning to wait until early next year before making any major changes at CNRS (and elsewhere) by which time he would have tested the water with the national colloquium on science and technology. The unions were unhappy with the CNRS directorate and had called for their resignation; but there was no sign of any movement yet.

Then, on Tuesday, after a long Socialist Party congress in which the party appeared to move to the left and chided the government for failing to confront the establishment more firmly, Chevènement decided he wanted to sack M. Christian Morrisson who was appointed director of social sciences at CNRS in April (beforé the general election).

Morrisson, it seems, was taking CNRS

social science in completely the opposite direction from Chevenement's own interests. The minister would like to expand social sciences in France, but in the direction of immediate public concern—such as unemployment, or the changes implied by new technologies. Morrisson's interests were more academic.

So Chevenement called Ducuing and asked him if at the council meeting due the following day he would propose that Morrisson be replaced by someone more sympathetic — a M. Maurice Godelier, professor of anthropology at the Ecole des Hautes Etudes. Ducuing asked for more time to consult his colleagues but was refused. He resigned, and the other resignations followed, on the principle that the minister's insistence was an interference with scientists' responsibilities.

Chevenement, however, did not seem too disturbed by the events, despite the cries of some that the resignations would throw out of joint his whole plan for the reorganization of science and technology in France. At a press conference on Wednesday he asked journalists why they were so excited about the affair, pointing out that he had only proposed one change whereas in America the whole top administration was turned over at a change of government.

The minister now has a clear field on which to place his men, well before he had expected the opportunity. He is likely to be quick to make new appointments, though they must be approved by the council of ministers.

Robert Walgate

their budget is in real terms not very different from what was agreed three years ago. Others say that little purpose is served in reminding one Administration of promises made by its predecessor.

One of the ironies of ISABELLE is that the problems of magnet design which have delayed the project by about four years appear in the past few weeks to have been resolved. If the Administration practises what it has been preaching the only remaining hope for the accelerators is that Congress may choose to appropriate funds for the project against the Administration's wishes

#### High-energy physics

#### LEP approved

While American physicists agonize over the future of a new accelerator (see above), twelve European nations last week agreed in principle to the construction of LEP, a 27-km circumference machine to collide electrons with positrons at energies up to 50 GeV per beam — enough to create the long-sought neutral intermediate vector boson.

The twelve nations are the members of CERN, the European centre for research on nuclear physics at Geneva where LEP will be constructed. Three members (the Netherlands, Sweden and Norway) have given approval subject to parliamentary approval at home.

The principal questions still hanging over LEP concern the precise annual budget at which it will be built — which affects how rapidly it might be brought into service — and environmental opposition in the French and Swiss territory under which the LEP tunnel must be bored.

If all goes well the budget will be decided at the December meeting of CERN Council. (On CERN's own plans, it would be about 630 million Swiss francs a year — £182 million — enough to have LEP in action by early 1987.)

But environmental approval is more unpredictable. At present, CERN is restricted from building even a reconnaissance gallery. The gallery is needed to permit inspection of the geologically critical boundary between the sandstone floor of the Geneva valley and the limestone Jura Mountains to the north (under which the LEP tunnel must pass), but a Lyons court ruled that CERN had no valid licence for the work. CERN has now guaranteed that the accelerator will not be built until and unless the appropriate local French and Swiss procedures for approving large constructions are completed successfully.

Such approval is yet to come — for the French and Swiss accession to LEP at the level of CERN Council was made at ministry level, and is subject to local approval. However, CERN staff are confident that the environmental "dangers" of LEP — for example that it might affect the water table — have been wildly exaggerated, and that local approval will be granted.

Robert Walgate

US research support

#### **Academy exhales**

Washington

Dr Frank Press's first essay as a constituency lobbyist since his election as president of the National Academy of Sciences, his colloquium on the latest budget proposals on Monday and Tuesday last week, was a tactical success. Its most tangible product, a document labelled "consensus statement", drafted in a closed session on the second day, is a nice amalgam of moderate belligerence and sympathy for the Administration's economic problems.

The colloquium itself was the first response of what the labour unions might call organized science to the decision in September that all federal agencies except the Department of Defense must reduce their discretionary expenditure by 12 per cent. (NASA, for the current financial year, is let off lightly at 6 per cent but has been told that 1983 will be worse). Discretionary expenditure is that not mandated by explicit provisions in legislation.

The consensus statement acknowledges that economic problems "have eroded research and development", but says that the new budget proposals will do "irreparable damage" unless long-term research is protected, if necessary at the expense of "development and demonstration". The document urges the Administration to assume responsibility for supporting scientific research, but asks for a formal review of the machinery by which this is done.

Before the definition of consensus preoccupied the colloquium, diversity was rampant. One speaker was so alarmed by the threatened 12 per cent reduction as to fear a return to pre-Sputnik days. Another warned his academic colleagues not to expect too much from industry, whose support now accounts for 3 per cent of university budgets. Industrial grants would have to increase threefold to make good the damage done by the 12 per cent cut.

The colloquium also broke new ground by giving currency to the word "prioritization" — deciding what research should be given first claim on limited funds. This notion is in sharp contrast with earlier declarations of faith in a plurality of sources of funds.

The statement argues that the abruptness of the new cuts will be especially damaging. This is well illustrated by the plight of the National Science Foundation, none of whose expenditure is mandated by Congress, but which finds itself committed to the US Navy for support of the Antarctic research programme as well as to several national laboratories. It has become known in the past few days that these commitments may mean that the softer, grantmaking parts of the foundation may find their budgets cut by up to 18 per cent, especially if spending on social science,

education and international relations (cut severely in March) is now protected.

Among the hundred participants the two principal government representatives appear to have left contrasting impressions on their audience. Dr J. Khaduri, one of the hard men from the Office of Management and Budget, preached economic realism and told his listeners that they would lose more from continued inflation





Press, moderately belligerent; Keyworth, inscrutable

than from the government's emergency budget. In the meantime, he declared, there is bound to be hardship. But at least there is also a chance that scientific enterprise could be growing again on the basis of a "new equilibrium" and with the help of dollars whose value remained constant from one year to the next. It is unfortunate that the Secretary of the Treasury, Mr Donald Regan, should have had to admit a few days later that the federal budget would probably not be balanced by 1984.

Dr George Keyworth's declaration seems to have been harder to interpret. The science community is not yet sure whether to regard him as a friend at court or as the Administration's lightning rod. Dr Keyworth himself is probably not yet sure. Last week, however, he left at least some of his audience with the impression that he believes the consequences of the emergency budget will be less serious than now seems likely. Does he know something, or is he simply being well mannered?

#### European space policy

#### New satellites due

Europe's activities in space received new impetus with the announcement last week that the United Kingdom is willing to make its contribution to the Large Telecommunications Satellite (LSAT-1), and with the decisions made at a European Space Agency (ESA) council meeting to recommend three projects to member nations for further development. Provided the member states do not refuse to fund them, the three projects - the Earth Resources Satellite (ERS-1), the Ariane 4 launcher and the Spacelab "follow-on" spacecraft - will begin their next development stages in about three months' time. Other issues of great concern to the members of ESA - particularly collaboration with the United States and the ten-year plan of ESA's director, Erik Quistgaard (Nature 290, p.536) - have been deferred until the next council meeting in December.

The Minister for Information Technology at the British Department of Industry, Mr Kenneth Baker, announced last Friday that the United Kingdom was willing to provide its 34 per cent share towards the cost of LSAT-1 (which would amount to about £77 million over the next ten years). The project will only proceed once other interested ESA members, particularly Italy, the Netherlands and Canada, have decided to fund it.

The concern of the United Kingdom has been that the balance of responsibilities for the specifications, management and marketing of the satellite, both before and after its expected launch on Ariane early in 1986, should be shifted from ESA to industry. The satellite will carry four groups of payloads, two of them primarily commercial, the others more experimental. The former are to be funded mainly by Britain and Italy, and are intended to provide efficient communications for businesses and facilities for high-powered direct broadcasting of television. The two more experimental payloads, involving Italy and Belgium, will function in the millimetre (20-30 GHz) waveband, and are intended to investigate signal propagation characteristics at these frequencies. Once the usefulness of LSAT-1 has been adequately demonstrated, further satellites of this type may be developed on a commercial basis and - to this end - the satellite has been designed to be launched by either Ariane or the space shuttle.

The decisions made at last week's ESA council meeting have increased the chances for launches of the Earth Resources Satellite, Ariane 4 and the successor to Spacelab in 1986 or thereabouts.

The successor to Spacelab will involve the development of increased electrical power and mission duration within the present Spacelab configuration. ESA's recommendation, if funded by member countries, will initiate the construction of this craft. Moreover, there will begin a study into the development of a pallet that would be launched from the shuttle to be retrieved from low Earth orbit later.

Ariane 4 is, to some extent, ESA's answer to the space shuttle, in that current models of Ariane cannot launch the large payloads that may be required over the period 1985-95. Modifications to the first stage, allowing an increased fuel volume and the addition of liquid fuelled boosters, will increase Ariane's maximum payload capacity to 4,300 kg (as opposed to Ariane 3's limit of 2,460 kg). It is intended that Ariane 4 will not only launch single large satellites such as Intelsat 6 but also provide an improved dual launch facility.

The oceanographic remote sensing satellite ERS-1 is now set for the second stage of its feasibility study, involving a detailed design and costing. Experiments already earmarked for the satellite will investigate the wind field and ocean wave

heights. An announcement of opportunity has also been issued for prospective experimenters to fill the remaining 50 kg of payload capacity. The final decisions on the mission's design and instrumentation will be taken in 1983. One problem still to be decided upon concerns the distribution of data from ERS-1, particularly the degree to which this is centralized.

Philip Campbell

#### Enrironment research council

#### Rothschild persists

The Rothschild customer/contractor principle, whereby British government departments (the customers) commission research in the research councils (the contractors) is still alive in environmental research, despite its demise in medical research and modification in agricultural research earlier this year. Its survival seems to be largely due to Sir Hermann Bondi, a well-known advocate of the principle, who has been chairman of the Natural Environment Research Council (NERC) for the past year.

Sir Hermann believes that the principle will work well, but only if NERC is careful to accept those contracts which will benefit its research as well as satisfying the customer. He also acknowledges the difficulties inherent in multi-customer contracts which can put a project at risk if one customer decides to withdraw support.

One such casualty is the £5 million Land Geological Survey of Great Britain which was threatened when the Department of the Environment, largest of the three customers, decided to cut its contribution for 1981-82 to two-thirds of its level in 1980-81 and to support only tactical research directed mainly at resource planning.

It plans a further cut in 1982-83. The result has been a substantial reduction in the amount of strategic and long-term research which is mainly geared to detailed geological mapping.

Despite these problems, however, Sir Hermann remains optimistic about the work of NERC. When introducing its annual report last week (HMSO, £4.00), he said that he had had a "marvellous time" during his first year as chairman. Although a slightly reduced budget has diminished the vigour with which the council can pursue some projects, he says he is impressed with the quality of the research it supports and sees no danger of stagnation.

During 1980-81, the year covered by the annual report, £26.1 million of the council's £72.9 million budget was earned from contracts, the remainder coming from the Department of Education and Science through the science vote. Support for universities, however, decreased slightly, with £75,000 less spent on new research grants than in the previous year (the council currently awards about £3 million of new research grants a year).

**Judy Redfearn** 

#### Hoechst in Boston

#### Contract cleared

Washington

Deft collaboration between the US General Accounting Office (GAO) and Congressman Albert Gore has now forced into the open the controversial agreement between Massachusetts General Hospital and Hoechst AG, the Frankfurt-based chemicals company. Earlier this year, representatives of the hospital declined to hand over their agreement with Hoechst to a congressional subcommittee of which Mr Gore is the chairman, on the grounds of commercial confidentiality. Under the agreement, Hoechst will support a new department of molecular biology at the hospital, spending more than \$50 million in the coming decade. In return, Hoechst will have the first refusal on an exclusive licence to exploit any patents generated by the laboratory during the period of its financial support.

The intervention of GAO, prompted by Congressman Gore, was based on the possibility of a conflict between the hospital's agreement with Hoechst and the requirements of the 1980 amendments of the patent legislation which, among other things, gave non-profit making institutions such as the hospital the right to first refusal of patent rights arising from federally supported research. The provisions of the new patent legislation came into force on 1 July this year, two months after the hospital's agreement with the company.

Evidently, the new agreement between Hoechst and the hospital has been drafted with the new legislation in mind. GAO says, in a letter to Mr Gore, that it should be possible for the hospital to keep separate the research funded by Hoechst and that funded by federal agencies, the National Institutes of Health (NIH) in particular. As things are, the hospital's research programme is supported to the tune of \$30 million a year by grants from NIH.

Difficulties will arise, according to GAO, only when there is doubt over whether a patent exploited by Hoechst has been supported exclusively by funds provided by the company, or where patents arising from jointly funded projects would be dealt with differently under the agreements between the hospital and Hoechst and NIH respectively. In all the circumstances, both the hospital and the company are likely to lean over backwards to avoid complications of this kind. Even so, when making public the terms of the contract on 16 October, Mr Gore uttered dark threats about the necessity that US institutions made strong with public funds should assist American and not foreign industry.

The details of the contract now published are unlikely to offend academic susceptibilities. Hoechst's support of the new molecular biology laboratory is defined by a programme of payments that will include the best part of \$18 million for

laboratory accommodation and equipment as well as \$50 million over ten years for running costs. Both sums are indexed against inflation, while the hospital administration will be allowed to charge an overhead percentage (within the \$50 million) equal to that agreed from time to time with NIH.

Under the agreement, the hospital is required to patent all inventions arising from sponsored work, but at Hoechst's expense. The company will be automatically entitled to an exclusive licence for exploitation, but the hospital will be able to take back the rights of exploitation if the company delays for more than three years: the agreement specifies that the director of the new department of molecular biology should be Dr Howard Goodman, who is already in post. One of the potentially contentious points in the agreement is that senior appointments be decided "after consultation with the company". The agreement also requires that those appointed should be "as appropriate" recommended for tenured appointments at Harvard Medical School.

On publication, the agreement requires that the company should be sent a copy of any proposed publication 30 days before this is sent off for publication, during which period the company will decide whether patentable discoveries are involved: all those employed at the department will be required to sign service agreements declaring that the hospital authorities will be notified of any possibly patentable discoveries. Collaboration with others is permitted, provided that Hoechst's exclusive patent rights are not prejudiced. Consultancy for other companies and organizations is permitted so long as there is full disclosure and discussion with the director of the department.

The agreement also defines the way in which the proceeds from patent exploitation will be shared between the inventors, their department and the hospital at large. The agreement says that royalty percentages negotiated should ordinarily be half those appropriate to commercial agreements, and that royalty income should be deductable from the annual payments to which the company is committed.

The agreement now published is very similar to the outline account of it given to the Gore committee earlier in the year, so it is not obvious why the hospital withheld it from the committee.

#### **SERC** director looks to the future

Britain must collaborate with its European neighbours if it is to have a stake in building major new research facilities in the future, according to Professor John Kingman who succeeded Sir Geoffrey Allen as chairman of the Science and Engineering Research Council (SERC) on 1 October. Precisely how big and costly a facility will be before international collaboration becomes worth while will depend on SERC's future resources and on the needs of potential collaborators. But if SERC was embarking now on some of the major facilities it agreed in the mid-1970s then international collaboration would almost certainly make sense, according to Kingman.

Indeed, the council is already looking for European partners to help with the construction of the spallation neutron source at the Rutherford Laboratory which, at an estimated cost of £15 million, is due to come on line during 1984. One possible collaborator is Germany which has considered building a similar facility of its own. But the council has also held discussions with other countries which may wish to use the facility.

SERC's difficulties over building major facilities began in the late 1970s when its budget failed to keep up with inflation, forcing it to lengthen construction times for major facilities and leading to an inefficient use of resources. Worst affected has been the nuclear structure facility at the Daresbury Laboratory which was originally due to come on line in 1978. Technical difficulties and a shortage of money at the right time have delayed its commissioning

until March 1982, although the capital cost (£13.5 million at 1980 prices) has kept roughly in line with inflation. The synchrotron radiation facility also at Daresbury, which was commissioned last June eighteen months behind schedule, has suffered a similar, but less acute problem.

Despite a static budget, however, SERC has had some recent successes. Kingman is particularly impressed with the work of the council's three directorates in encouraging collaboration on engineering research between academics and industry. The council set up its fourth directorate in biotechnology last week (see this page) but Kingman is doubtful that it can afford to set up a fifth in microelectronics unless it can transfer responsibility for those in marine and polymer engineering to industry in general or the Department of Industry in particular.

One of Kingman's major problems will be how to maintain the quality of science in British universities which are suffering an unprecedented cutback in income. Although he is sceptical of government promises to maintain the real value of the science vote, he says that he is determined to maintain spending on research grants and studentships at least at its present level. That could mean convincing the Advisory Board for the Research Councils, which divides the science vote between the five research councils, that SERC should have a larger slice of the cake. It will also mean maintaining numbers in the face of overall cuts in research studentships already made by the Department of Education and Judy Redfearn Science.

#### UK biotechnology

#### Still striving

The British Science and Engineering Research Council (SERC) last week launched a new directorate to foster collaboration in biotechnology between academics and industry and to forestall any brain drain of British biotechnologists to greener pastures abroad. The new biotechnology directorate is partly a response to a major study, chaired by Dr Alfred Spinks, which recommended nearly two years ago that Britain must act swiftly if it is not to lose out on the commercial development of biotechnology. Contrary to appearances, however, SERC has not been tardy in its response, according to Dr Geoffrey Potter. who will lead the new directorate. SERC's specialist panel on biotechnology spent the past year working out precisely what to do.

The biotechnology directorate will perform a function similar to the existing SERC directorates in polymer and marine engineering except that it will report to both the science board and the engineering board, reflecting the broad spectrum of research that biotechnology encompasses. One of its most difficult tasks, according to Dr Potter, will be to motivate process engineers, notoriously more reluctant than microbiologists to seize opportunities in biotechnology.

The directorate's funds will only be modest, £1 million this year rising to £2.4 million by 1984-85. The extra money will come from the Advisory Board for the Research Councils and from economies in SERC's other activities.

Most of the money will be spent on fostering collaboration through schemes already used by SERC to get industry involved in research in universities. These include the teaching company scheme and Cooperative Awards in Science and Engineering (CASE), both of which support postgraduate students on research projects relevant to collaborating companies, and the cooperative grant scheme whereby SERC and collaborating companies chip in to the cost of research projects in university laboratories.

SERC is particularly keen to encourage collaboration on fermentation, enzyme and immobilized cell technology, separation and concentration technology, product processing and recombinant DNA research. The directorate is to work closely with the Department of Industry which may take over funding of projects approaching the development stage, and with the Agricultural Research Council and Medical Research Council, both of which also support biotechnology.

One of the directorate's aims, according to Dr Potter, is to create sufficient jobs to dissuade British biotechnologists from taking posts in industry and universities abroad and even to persuade those who have already left to return. Dr Potter's concern about a possible brain drain is

shared by the members of a Royal Society working party, chaired by Professor W.D.P. Stewart, which will shortly publish a report on biotechnology and education.

The working party estimates that over the next ten years Britain will need 1,000 extra graduates and 4,000 technicians trained in biotechnology. It does not, however, favour new undergraduate courses specifically in biotechnology; training should insted be based on existing undergraduate courses in biology and chemical engineering followed by more specialized postgraduate courses.

The working party also supports a recommendation originally made in the Spinks report that the University Grants Committee should create 20 new lectureships in selected universities. That request, together with many of the Spinks recommendations, received short shrift from the government, which said in a White Paper earlier this year that the development of biotechnology in Britain should depend on market forces rather than government intervention.

Judy Redfearn

#### **Hungarian protests**

The use of psychiatric methods to treat political dissent has reappeared in Hungary for the first time in more than a decade, with the confinement in a Budapest mental hospital of Dr Tibor Pakh, a 57-year-old lawyer and activist from 1956. Dr Pakh's hospitalization evoked a sharp letter of protest from more than 50 Hungarian intellectuals and scholars.

Since the rise of Solidarity, Dr Path has issued a number of open letters supporting the Polish liberalization and censuring the Hungarian authorities for echoing Moscow's condemnations of it.

On 4 October 1981, Dr Pakh attempted to travel to Poland. He was stopped at the Hungarian frontier, his passport and personal papers were confiscated, and he was forced to return to Budapest. On 6 October, when his protest to the Procurator General's office failed to obtain satisfaction, he began a protest fast in the University Church in Budapest. Three days later, he was forcibly conveyed to hospital, and given intravenous feeding and heavy doses of psychotropic drugs including haloperidol, one of the drugs used in similar cases in the Soviet Union.

The group of intellectuals who signed the protest letter maintained a constant stream of visitors to the hospital, demanding to see Dr Pakh and also forwarded their protest to Solidarity, who published it in their uncensored bulletin *Niezaleznosc*. The protesters apparently made their point, and on 26 October, Dr Pakh was released, ostensibly on "readjustment leave".

Vera Rich

#### Interferon

#### Gamma winners

Molecular biologists at the Californian biotechnology company of Genentech have won the race to clone a sequence of DNA corresponding to  $\gamma$  interferon, the least understood member of the family of proteins which may yet find a place in the therapy of cancer and viral diseases. At the same time Molloy Laboratories, a subsidiary of Revlon, have been contracted by the US National Cancer Institute (NCI) to purify sufficient  $\gamma$  interferon from natural sources for initial clinical trials.

The Genentech results, briefly presented by Dr David Goeddel at the Second Annual International Congress for Interferon Research in San Francisco, not only establish the sequence of y interferon but also show that bacteria, yeast or mammalian cells are able to produce y interferon when supplied with the corresponding sequence of DNA.

The starting material for both Genentech and Molloy was human lymphocytes, prime producers of y interferon. From them Genentech isolated a mixture of messenger RNA molecules, produced the complementary DNA molecules, and transplanted them into cells of the bacterium Escherichia coli. Bacteria were then isolated which were producing the antiviral activity of interferon but with the instability towards acid that distinguishes y interferon from the  $\alpha$  and  $\beta$  varieties. Finally the DNA responsible for that activity was sequenced and shown to be about the same length as that of a interferons but with an unrelated sequence.

Human lymphocytes are also the starting material for the y interferon that Molloy, in return for \$270,000 from the NCI, are to produce by traditional methods of purification from normal white blood cells obtained as a by-product of blood transfusion. Their aim is to produce five billion units of y interferon, enough for up to 1,000 human doses, by the end of September 1982.

It is a matter of speculation whether the Genentech and Molloy materials will be equivalent. A lot depends on whether there is a single  $\gamma$  interferon or whether, like  $\alpha$  interferon, it is a family of related molecules. Genentech has no evidence of more than one species but if they do exist they are likely soon to be discovered either by the Genentech scientists or by those whom they beat to the first sequence, including Dr Charles Weissmann on behalf of Biogen, Dr Jan Vilcek of New York University Medical Center and Dr Leroy Hood of the Californian Institute of Technology.

The hope of all concerned is that y interferon will be of greater value than its stablemates in the therapy of cancer. The hope stems from the fact that antitumour effects of interferon are thought to work through the immune system and that y interferon is produced by and has effects on cells of that system. Reasonably large clinical trials of  $\alpha$  and  $\beta$  interferon against cancer and viral diseases are currently under way using material purified from cells. It will be some time before their value is clear and even longer before it is known if  $\gamma$  inteferon is more effective.

Peter Newmark

#### Royal Botanical Gardens

#### Look to the margins

This week sees a new man in charge of the Royal Botanical Gardens at Kew in London — Professor Arthur Bell, a biochemist, formerly head of the department of plant sciences at King's College. And it could mean a very different approach for an institution with a 140-year history of traditional botany behind it.

The Royal Botanical Gardens today include Kew together with a 600-acre estate at Wakehurst Place in Sussex, and are run as a department of the Ministry of Agriculture, Fisheries and Food with a scientific staff of almost 500. The emphasis is still very much on the traditional pursuits



Bell in situ at Kew

of collecting wild plant species (Kew boasts the world's largest herbarium) and taxonomy. Kew's new director, however, brings with him an enthusiasm for plant breeding not seen there before. And Professor Bell has a clear goal in view — to change the pattern of agriculture in the Third World.

At present just thirty plant species provide eighty per cent of the world's food supply. Many of these basic food crops are now grown in arid conditions far removed from those in which their free-growing ancestors used to thrive. Professor Bell's contention is that there are many indigenous crops used only as animal fodder, or unpalatable because of the presence of toxins, which would give better yields than today's food crops if suitable variants were selected. Therefore much of Kew's effort will centre on isolating variants of these so-called "marginal" crops which do not produce toxins, but which still grow well in arid climates. In this way perhaps Kew will again become the important source of new crops that it once Charles Wenz was.

#### CORRESPONDENCE

#### **Brazilian funds**

SIR — I would like to correct some of the statements made by Mr Bazin in his article "Brazilian research funds: Sliding backwards" (Nature 6 August, p.489).

First, the Brazilian government has a clear and explicit policy statement regarding science and technology. This was published in the form of the 3rd Basic Plan for Scientific and Technological Development (1980–85) and stated in the 3rd National Development Plan.

Second, the Brazilian government, in harmony with the high priority given to science, has maintained a steady and growing support for scientific and technological activities. Financial resources allocated to science and technology in the federal budget are shown in Table 1.

Table 1 Federal support for science and technology

	Science and technology budget	% Of
Year	(US\$ million)	federal budget
1980	352	2.1%
1981	435	2.3%
1982	909*	3.6%

\*Proposal now in discussion in Congress.

Third, the National Research Council (CNPq) which is the central coordinating agency for science and technology in Brazil, has had and has now an important role to maintain and reinforce this growing public

Table 2 CNPq's budget

Year	Total budget (US\$ million)	% Allocated for grants and scholarships
1980	122	31.9%
1981	144	33.7%
1982	188*	38.0%
*Proposal		

support for research in the country. The data in Table 2 show that CNPq's budget, as well as the resources allocated for grants and scholarships, have outpaced inflation.

ERNO IVAN PAULINYI

CNPq, Brasilia, Brazil

MAURICE BAZIN REPLIES — When considering whether Brazil's research spending has kept pace with inflation it should be remembered that inflation in Brazil was over 80 per cent in 1980 and is now around 110 per cent.

#### Gold standard

SIR — The reexamination of the gold standard in the United States is motivated by more serious concerns than your article on the subject suggested (*Nature* 24 September, p.246). For, despite its faults, the gold standard performed an essential function for which no other arrangement has so far been able to substitute in a democracy. It provided an impartial mechanism which the general public accepted as justification for the monetary discipline necessary to prevent accelerating inflation.

From 1717, when Sir Isaac Newton established the official buying and selling prices of gold at 77/9d and 77/10½d per troy ounce, until the outbreak of the First World

War in 1914 (apart from a period around the Napoleonic Wars), a pound sterling was worth ¼ ounce of gold (within 1 per cent), and it was universally accepted that the Bank of England should maintain adequate reserves of gold to guarantee sterling's convertibility. Thus, when official gold reserves fell below a prudent level, the steps necessary to increase them, including in particular the raising of interest rates, were accepted by the public (and the parliamentary opposition) in preference to the abandonment of the gold standard.

By contrast, since the last link with gold was broken in 1971, there has been no economic "yardstick" which is publicly accepted as an objective measure of sound monetary policy, and periods of economic difficulty are blamed on the incumbent government rather than on our own behaviour. Politicians have inevitably tried to "buy" popularity, and as a result, in only ten years, sterling has been devalued by 75 per cent in real terms, at terrible economic and social cost to the United Kingdom. The United States is surprisingly close behind.

There is, in fact, a very great need to link currency to a real measure of value in a system of which the soundness and impartiality are beyond question and clear to both public and politicians. However, the standard should not be some "short-lived exotic radioactive isotope" as you suggest, but should be a range of the durable, essential, basic commodities on which all economic activity depends. The many far-reaching advantages of such a policy have been described by a number of economists over the past century, but the specification of a practical system is complicated by the need for commodity prices to respond to market forces; they cannot be fixed as was the price of gold. Nevertheless it would be possible to achieve the desired results by establishing a rule by which official buying and selling prices of specified commodities were adjusted automatically according to the levels of official reserves. Such a system has been widely endorsed by economists from across the political spectrum, one writing in 1958: "It can be only a question of time before man's reason and self-interest overcome his inertia and these proposals are accepted. When they are they will define the beginning of an era as surely as did the introduction of the gold standard, but without its fatal weakness"

Institutional resistance to change has so far prevented the adoption of such an innovation. But until we do, inflation will continue, with all its destructive consequences (and economics will continue to be as unscientific as physics would be without the ruler).

P.Q. COLLINS Imperial College of Science and Technology, London, UK

#### Lab animals

SIR — Your article on "Protection for laboratory animals" (Nature 17 September, p.173) offers an ineffectual solution to the problem of how to regulate the use of animals in research. Your suggestion of establishing a committee at each institution empowered to sanction proposed uses of laboratory animals seems like an attempt to eliminate regulation entirely. No research facility would refuse to sanction the use of animals for an experiment in which funding has been previously granted.

For regulation to be effective, it must be done by the agency responsible for funding.

Ignoring humane and philosophical considerations, the need for regulation is generated by the sheer numbers of research facilities in existence today. In order to prevent wasteful duplication of experiments, a coordinating agency must be empowered to determine the usefulness of a particular project. Since public monies are usually involved, the usefulness and more importantly the uniqueness of a study must be determined. Of course, a certain amount of replication of experimental results is necessary, but as there is virtually no communication between facilities, a parent agency must be involved to keep track of who is doing what.

We have a responsibility to the animal community, from which we have gained much information at the expense of much suffering, to use individuals as prudently as possible. Whether or not you believe an animal is capable of suffering in human terms is not important. It is a living entity, entitled to the life it was designed to lead.

Regulation of the use of animals in research experiments is essential to assure the proper use of public funds as well as the compliance with laws governing the humane treatment of the subjects. A governmental body, specifically the agency responsible for funding of research projects, must be responsible for the regulations.

Jackie Alan Giuliano Jet Propulsion Laboratory/California
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SIR — I was pleased to see your editorial comment "Protection for laboratory animals?" (Nature 17 September, p.173). The Committee for the Reform of Animal Experimentation (CRAE), which is drawn from both houses of parliament and from the fields of science and medicine as well as animal welfare, has been seeking contact with members of the scientific community at all levels in order to find a "middle ground" where there can be agreement on many of the issues relating to the use of living animals for research. We would be very pleased to hear from those working with animals in laboratories if they have any points of view to express.

CRAE applauds your proposal for the setting up of ethical committees within research centres to act as a brake on undesirable experimentation. Such committees should be largely composed of researchers' peers but should also include what the courts describe as "the reasonable man", that is, responsible members of the local community.

The concept of ethical committees would fit very well with the Home Secretary's Advisory Committee proposals for a project licence as a requirement as well as a competence licence before a researcher could experiment.

One error in your editorial: although the 1876 Act requires experiments involving pain to be carried out under anaesthesia, such provision is the subject of exemption by the issue of Certificate "A" which permits the infliction of either severe or enduring pain, and it is only where an animal is found to be suffering severe and enduring pain that the experiment must be terminated.

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#### NEWS AND VIEWS

# Integration of foreign genes into the mammalian germ line: genetic engineering enters a new era

from Brigid Hogan and Jeffrey Williams

THE application of recombinant DNA technology to mammalian cells is gradually yielding a rich harvest of cloned chromosomal fragments containing specific genes and their flanking sequences. Some of these cloned DNAs have been reintroduced into tissue culture cells where in many cases they are efficiently expressed. However, these experiments are of little value when it comes to identifying DNA sequences which control the temporal and tissue-specific expression of genes during embryonic development. To study these problems, a number of laboratories have injected cloned DNA into early mouse embryos and seen if the genes become stably integrated into the

It was hoped that the injected embryos would grow into adults, each carrying the same exogenous DNA inserted into different chromosomal sites in their eggs or sperm. Offspring of these individuals would then generate mouse substrains with the inserted DNA in all cells right from the start of development, allowing the effect on gene expression of such factors as neighbouring sequences, chromatin structure and DNA methylation to be assessed. The results of these experiments are now being reported in varying degrees of detail, and the consensus is that the bold approach has paid off, with the process of chromosomal integration being more efficient than people had dared imagine.

Support for the idea that cloned genomic DNA could be inserted into the germ line via the early embryo came from the work of Rudolf Jaenisch and his colleagues1-3 on the stable integration of exogenous Moloney leukaemia virus (M-MuLV). When 4-16 cell or blastocyst stage mouse embryos are infected with virus and then implanted into foster mothers, around 10-40 per cent per cent of the adults show active virus production in various tissues. Of these animals, a high proportion of the males can be shown to have M-MuLV DNA integrated into their germ line, since on mating with normal females they produce male offspring which transmit M-MuLV to 50 per cent of the next

generation (only males are studied, to avoid problems of in utero transmission of virus). The original virus-producing males developing from infected embryos show only a variable and declining transmission of M-MuLV. There are at least two reasons for this. First, the virus may only infect a few of the embryonic cells, giving rise to an adult in which the tissues are a 'mosaic' of cells with or without integrated virus. Second, spermatogenic cells carrying M-MuLV may be selected against in the mosaic testis as the animal ages. However, the second generation M-MuLV positive males carry viral sequences in all somatic cells, and transmit the DNA as a normal, dominant, gene to 50 per cent of their offspring. Using the technique of embryo infection, Jaenisch has generated thirteen different mouse substrains (Mov 1-13) carrying M-MuLV in specific sites. Study of these substrains has led to the exciting observation that integration of virus into different sites results in different temporal and tissue-specific patterns of viral

In Mov-1 mice, for example, virus production is first seen in the spleen soon after birth, while in Mov-13 mice virus activation occurs much earlier, in liver cells after only 16 days gestation. In other substrains, virus may never be activated during normal life. The pattern of M-MuLV expression is therefore influenced by the chromosomal site into which the virus is integrated.

The converse of this effect may be the influence of MuLV sequences on the expression of genes into or near which they are inserted. Recent studies from the Jackson Laboratory in Bar Harbor<sup>4</sup> have shown that the *dilute* coat-colour mutation in DBA-mice is linked to an endogenous MuLV sequence, and that spontaneous revertants from *dilute* to wild-type (d to  $d^+$ ) have also lost the viral sequence. It is therefore possible that the original d mutation, as well as other coat-colour mutations, are the result of provirus

Brigid Hogan and Jeffrey Williams are in the Imperial Cancer Research Fund, Mill Hill, London. integration into the genome.

In the experiments of Jaenisch and his colleagues described above, infection of preimplantation embryos led to mosaic adults composed of cells either lacking integrated virus or with virus integrated into different sites. However, more recent experiments5 have shown that if MuLV DNA cloned from Mov-3 animals is iniected into the cytoplasm of a fertilized egg. viral sequences can be found in all of the cells of the resulting adult. The integration does not appear to be direct, however, and is not into the Mov-3 site. Rather, it appears that the DNA is first transcribed and then reintegrated into a new stable site in the zygote genome. This method therefore provides a quicker way of studying the effect of chromosomal integration site on viral expression, and of inserting M-MuLV DNA into the germ line. Unfortunately, the answer to the problem of how to direct M-MuLV into a defined site remains elusive.

Laboratories attempting to insert functional chromosomal genes into the germ line have also tried injecting cloned DNA into the fertilized egg, although in these cases the DNA is actually injected into one of the two pronuclei before they have fused. The first reports to appear<sup>6,7</sup> showed that recombinant bacterial plasmids injected in this way were retained in some of the surviving animals in a high molecular weight form. The amount of DNA present was sufficient to suggest that most, if not all, of the cells contained multiple copies of the plasmid. Two obvious questions arise. Has the foreign DNA been recombined into the chromosomes and, if so, does the germ line contain integrated DNA? Both of these questions are now elegantly answered in experiments published in this issue of Nature (see p.92) by Constantini and Lacey of the Department of Zoology, Oxford. They injected a \( \lambda \) recombinant containing the adult rabbit  $\beta$ -globin gene and a  $\beta$ -like pseudogene  $\psi\beta2$  in a 19 kilobase chromosomal DNA fragment. Partial hepatectomies were performed on 24 of the mice, and total liver DNA screened for the

presence of rabbit  $\beta$ -globin genes by Southern blot hybridization. Nine of the mice contained rabbit  $\beta$ -globin genes in copy numbers varying in different individuals from one or two to up to twenty per cell although further experiments are now needed to establish whether the animals are mosaics, with some cells containing more copies and others none.

Four of the positive males were mated with normal females and DNA from the progeny screened for the presence of rabbit globin genes. Out of a total of eighteen progeny screened, six were found to contain rabbit globin sequences. This pattern of inheritance is consistent with a chromosomal location of the foreign DNA and, in a footnote to their paper, Constantini and Lacey describe the results of an in situ hybridization experiment showing that most, if not all, copies of the rabbit globin genes in one of their mice are located in the middle of one of the homologues of chromosome 1. The organization of the rabbit  $\beta$ -globin gene copies has been determined in a number of the mice by restriction mapping with various enzymes. Predominantly linear molecules of  $\lambda$  DNA were injected, but the integrated DNA contains the fusion fragment resulting from annealing of the  $\lambda$ cohesive ends. Consequently, the authors suggest that the input  $\lambda$  DNA is ligated to form high molecular weight concatemers and it is these tandem arrays which are then integrated into the chromosome. There is direct evidence that such a mechanism occurs for linear DNA injected into fertilized eggs of Xenopus laevis 8. Also Constantini and Lacey detect minor fragments which are transmitted to progeny mice, and which probably contain the junction between the terminal copies of the foreign DNA oligomers and host chromosomal DNA sequences.

What then of the questions posed at the outset? Are foreign genes which have become stably integrated into the mouse genome expressed, and do they display the correct temporal and tissue specific pattern of gene expression? A newly published study by Wagner and his colleagues at Ohio University, describing work done in collaboration with Hoppe at the Jackson Laboratory suggests that at least the first of these two important goals has been achieved9. A proportion of mice, derived from eggs which have been injected with a rabbit  $\beta$ -globin gene were found to contain a rabbit globin polypeptide in their erythrocytes, and this was also true of their progeny. Furthermore, a recent report in Science10, quotes preliminary evidence

obtained by Wagner which indicates that the rabbit globin polypeptide was produced only in red blood cell precursors and not in other tissues of the mouse. If this latter observation is confirmed, the way would seem to be open to determining those features of DNA structure important in controlling gene expression during development. Also, of course, if it has indeed been possible to insert a cloned gene sequence into the mammalian genome so that it functions correctly, the wider implications are enormous. Leaving aside the controversial question of gene therapy in humans, it seems certain that the genetic manipulation of agriculturally important animals will quickly follow, since, in cattle at least, the techniques of superovulation and reimplantation in foster mothers are now routine procedures.

#### Extraterrestrials where are they?

from Ben Zuckerman

IN THE MIDST of a sometimes depressing reality, many people take conscious or subconscious comfort in the assumption that we are but one of a myriad of advanced technical civilizations co-existing in our Milky Way galaxy. This view championed by articulate scientific and lay spokesmen — is bolstered by lavish science fiction movies, by reports of UFO encounters of the first, second, and third kinds, and by best selling books about artefacts left behind by so-called ancient astronauts

Included among the articulate scientific spokesmen is Carl Sagan of Cornell University, whose television programme and book Cosmos have brought astronomy into the homes of millions of Americans. Sagan, and some other well known scientists such as Philip Morrison (MIT) and Frank Drake (Cornell University), are considered to be members of the 'optimistic' school of thought on the question of extraterrestrial intelligence. They have argued that 100,000 or more technical civilizations, all of which have arisen independently, may currently exist in our Milky Way galaxy. If there are this many civilizations, then a typical one must exist for a million years or more.

How do the optimists arrive at this value for N, the number of technical civilizations in the Milky Way? Usually they rely on the 'Drake equation' which expresses N as a product of the various probabilities of the origin and evolution of life, intelligence, and technology. Sagan went through this exercise in Cosmos. He supplied his estimates for a long string of uncertain factors including, for example, the probability, P(life), that life will originate on an Earth-like planet and the probability that, having originated, it will then evolve to intelligence and technology. Since we really know very little about these probabilities, the optimists rely on the Copernican world view that we are not special. After all, most Europeans once believed that the Earth was located at the centre of the Solar System. After that was shown to be false, it was still commonly believed that the Sun was located at or near the centre of the Universe. As this now also seems most unlikely, the optimists argue, so should be the view that intelligent life is unique to the Earth given that there are hundreds of billions of stars in the Milky Way and most of them have been shining for at least five billion years.

This world view compels them to choose very large values for the probabilities mentioned above. For example, many (most?) scientists would regard P (life) as uncertain by a factor of at least a million. Yet Sagan, in Cosmos, purports to know P(life) to two significant figures. His choice of  $P(\text{life}) \sim 0.5$  is, ostensibly, the result of taking the arithmetical mean of unity, which he prefers, and zero which is preferred by some of the pessimists in this field. Of course, he would have obtained a very different value for N if he had instead set P(life) equal to zero, the geometrical mean of 1 and 0. This illustrates the kind of tricks that one can play with the Drake equation.

Until the past few years, there were very few scientists who would argue strongly against this Copernican philosophy. Ten years ago, when I first became seriously interested in this problem, I was sufficiently uncertain of the value of N that I attempted to search, with Patrick Palmer (University of Chicago), for radio signals from another civilization. We pointed the 300- and 140-foot telescopes of the US National Radio Astronomy Observatory towards approximately 670 nearby stars. Since then half a dozen similar attempts have been made by radio astronomers in the United States and Canada. None of these has been successful, unless someone is hiding something in their bottom drawer.

Radio beacons are, of course, but one of many ways that advanced technological societies could make their presence known to scientists here on Earth. The most obvious way would be for the extraterrestrials to send a manned or unmanned probe to our Solar System and, especially, to land one on Earth. That there is no real evidence that this has ever occurred has suggested to Michael Hart (Trinity University) and others that

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advanced civilizations do not exist anywhere in our galaxy. We may well have the most advanced brains in the entire Milky Way.

At first glance it would seem to be a preposterous extrapolation to jump from the fact that we have not yet uncovered any evidence, either in deep space or in our Solar System, for superior extraterrestrial beings to the conclusion that they don't exist anywhere in the Milky Way. After all, our radio searches have been quite primitive and one can think of lots of reasons why another civilization might not want to go to the trouble and expense of sending complex probes between star systems.

This argument is reasonable if we are trying to understand why only a few civilizations might not want to send out interstellar spaceships. But what if there have been 100,000 or more civilizations, each of which must have existed, on the average, for a million years or more? Just as life and now, especially, intelligent life has transformed the face of the Earth so it might reasonably be expected that most of these 100,000 superior civilizations will have carried out deeds that, at present, we would regard as technological 'miracles'. Suggestions have included colonization and reengineering of the moons and planets of the home solar system to colonization and reengineering of the entire galaxy — all on time scales much less than 10 billion years.

Discussion of the many implications of these arguments filled an entire symposium whose proceedings, Extraterrestrials — Where Are They? are scheduled for publication by Pergamon Press in the autumn of 1981. Papers were presented by venerable pundits including, for example, Freeman Dyson (Institute for Advanced Study), Sebastian von Hoerner (National Radio Astronomy Observatory), and Ronald Bracewell (Stanford University) as well as by various comparative newcomers to this field.

Although many scientists find the arguments that N must be a very small number ( $\sim$  unity) quite compelling, few, if any, believe them to be so compelling that we should abandon searches for radio or infrared radiation from extraterrestrial beings. It is, therefore, ironical that Senator William Proxmire (Wisconsin) has entered these arguments into the Congressional Record of 30 July 1981 as part of his justification for prohibiting the use of federal funds during fiscal year 1982 for a modest radio search programme under consideration by NASA's Ames Research Center and the Jet Propulsion Laboratory. (He also felt that NASA wanted to search in the wrong place: "I have always thought that if [our best scientists] were going to look for intelligence, they ought to start right here in Washington. It is hard enough to find intelligent life right here. It may even be harder, I might say, than finding it outside

our Solar System.")

A few years ago Proxmire succeeded in deleting federal funds earmarked for a similar NASA search programme and gave NASA one of his Golden Fleece awards. Frank Drake, in return, gave Proxmire honorary membership of the Flat Earth Society but all to no avail. NASA has attempted to keep the programme alive, albeit at a minimal level, by using discretionary funds but the recent congressional action has stopped even that, at least for 1982.

This decision seems unfortunate for two reasons. First, there is considerable public interest in the search for intelligent life, judging, for example, from the response to Cosmos and the growing popularity of courses on "Life in the Universe" on college campuses. Second, and more generally, is it really in the best interests of American science for individual Congresspeople to fiddle with relatively minor items (about a million dollars a year) in the much larger budgets of major institutions such as NASA? Or, in the absence of a congressional vote of 'no confidence' in the NASA leadership, shouldn't the latter group, in conjunction with their scientists and engineers, be the ones who determine expenditures at the megadollar level?

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# Histone gene organization: paradigm lost

from Larry Kedes and Rob Maxson

THE organizations of sea urchin and Drosophila melanogaster histone genes were shown, even before the era of gene splicing, to be remarkably similar: both are repeated in the hundreds and arranged in tandem units, each unit containing the intimately linked genes for five major histone proteins. While the differences between the sea urchin and the fruitfly histone gene topology hinted that the order and polarity of the genes within a species can be gently shuffled, the similarity between species so widely separated in evolutionary terms seemed to establish a paradigm for histone gene arrangement and lead to the confident expectation that similar arrangements would be found in intervening phyla.

However, a rash of recent reports of different patterns of histone gene organization in a number of different vertebrate species, including examples of scattered and often solitary genes, has dispatched classical notions about their fixed organization. The authors responsible for the new observations have been quick to point out the difference between the more familiar sea urchinfruitfly tandem organization and the vertebrate dispersed topology and to speculate on its significance. Human<sup>1-3</sup> mouse<sup>4</sup>, chicken<sup>5,6</sup> (see this issue of Nature, p.49) and toad7-9 genes have now been cloned. Not only are the genes differently arranged in comparison with the classical paradigm, but they seem scattered and separated by long stretches of non-histone DNA (for an extensive review see ref 10). While a few coding regions remain clustered, they are not at all arranged in clean-cut tandem repeats. Some coding regions are solitary and separated, by at least 10 to 20 kilobases, from their fellows. None seem to be an

identical copy of any other suggesting that dispersion allows, or at least associates with, evolutionary drift in gene sequence. Each coding region seems to be present only in tens per haploid genome. How close the histone gene members of this newly discovered diaspora are to one another in the genomic landscape has yet to be determined. An earlier report<sup>11</sup>, using *in situ* hybridization, suggests that the human genes may be landsmen: the locus seemed confined to the telomere of chromosome 7 but the genes are scattered over the entire distal third of the long arm.

Recent findings in our own laboratory suggest that the distinction between clustered and dispersed histone gene organization is not a strict interspecies difference. As pointed out by a number of workers, a gradual shift in expression occurs during early sea urchin development: the activity of the several hundredfold tandem clustered 'early' gene set is reduced in favour of the expression of a set of 'late' histone genes of much lower copy number. Some features of late sea urchin histone gene organization have now come to light with the cloning of several examples12. The late genes are scattered and shuffled not unlike those described for the vertebrate examples mentioned above. Genes coding for isoforms of the same histone exhibit extensive nucleotide sequence divergence. And to complicate matters, not all of the vertebrate genes follow the same model: histone genes in the newt12, Notophthalmus viridescens, share all the topological features of the sea urchin and fruitfly clustered arrangement except that each repeat unit is separated by a long

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stretch of satellite DNA.

Evolutionary models for the establishment of tandem gene clusters either from dispersed gene duplications, or by dispersal of gene elements from a tandemly amplified gene family, seem to be equally possible. Isolated dispersed members of tandem gene families14 have definitely been found among sea urchin and Drosophila histone gene families and among mouse alpha-globin genes<sup>15</sup>. Thus, mechanisms do exist that allow copies of gene regions to be banished to remote corners of the chromosomal countryside where they can diverge and possibly take on a new function or fall prev to new regulatory controls. Such a scenario may represent the mechanism that generated dispersed late sea urchin histone genes from the repetitive early cluster. Catastrophic deletion of an amplified cluster following speciation could account for the absence of tandemly repetitive histone genes among most vertebrates.

Equally convincing evidence may exist on the side of an explanation that holds that scattered organization predates the amplified cluster of genes. The yeast, Saccharomyces cerevisiae, has H3 and H4 genes head to head at one locus and H2B and H2A lying at another locus in the same posture<sup>16-18</sup>. Both loci are duplicated so only a dispersed organization exists in this protistan. Simple amplification by gene duplication and unequal crossing-over within one of the scattered clusters of histone genes may be the organizing principle that might generate a tandem cluster. There are those who correctly point out, however, that yeast is a simplified evolutionary descendant of more complex protista and that an examination of histone gene organization in algae or slime moulds, for example, might better establish the validity of this model.

These observations about apparently chaotic principles underlying the alternative arrangements of histone genes can hardly be considered as a "paradigm regained" but they underline the plasticity and organizational variability of functionally related regions of the eukaryotic genome and point out that we will continue to be surprised by further revelations about the degree of such diversity and mechanisms of generating it.

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#### Poznan: Polish palaeohydrology presented

from Peter D. Moore

PALAEOHYDROLOGY is a topic which impinges upon many scientific disciplines. Physical geography, geomorphology, climatology and archaeology can all contribute to an understanding of why changes occurred in hydrological cycles in the past. Dry valleys, river palaeomeanders, the development of certain types of peat deposit, all bear witness to past conditions which may differ from those now found. To decipher such fossil geomorphological evidence and to reconstruct the hydrological conditions of past times could assist in the understanding of palaeoclimatic changes and help in seeing just how far reaching was the influence of prehistoric man upon his environment.

Poland has a long and distinguished history of research in this area and it is fitting that Poznan in Poland should form the centre for a recent conference on the palaeohydrology of the temperate zone.

L. Starkel (Krakow University) set out the complexities of this subject by showing how many varied factors have influenced the fluvial palaeoenvironment, including ice melt, sea level variation, tectonic effects and human impact. All of these have left their mark upon river patterns and sediments, thus making it difficult to evaluate the role of climatic changes. The late Pleistocene saw a change in river patterns from braided to meandering systems, which reflects lower fluvial activity following the final melting of ice and establishment of vegetation, but the trend has been interrupted on several occasions. Starkel claims that phases of increased fluvial activity recorded by such characteristics as increased grain size in fluvial deposits, more rebedded pollen and faster sedimentation rates, are synchronous over northern Europe. Periods of generally increased flood frequency occurred at 8,500-8,000, 6,800-5,800, 5,000-4,500 and 2,800-2,000 BP. All these, he claims, correspond to times of high lake levels and glacial advance, which supports the case for an overall climatic control. The increase in braiding pattern development in recent centuries, however, can be related to human activity.

It is very difficult, nevertheless, to demonstrate that any such effect is indeed purely climatic in origin. Very frequently, as discussed by K.J. Gregory (University of Southampton) and B. Frenzel (University of Stuttgart), one can detect evidence of increased human activity at precisely those times when palaeohydrological changes involving increased fluvial activity have

Petes D. Moore is in the Department of Plant Sciences, King's College, University of London. taken place. Forest clearance involves the reduction of transpiration rates in the vegetation and also lowers the interception of precipitation, thus permitting larger volumes of water to move through catchments into the rivers. Such evidence is often circumstantial, but its presence lays a considerable onus of proof upon those who would assert that purely climatic factors underlie the observed river changes.

Circumstantial evidence on one side can, of course, be countered by similarly based arguments on the other side. If widespread climatic changes can be demonstrated, which coincide with the fluvial palaeoevent, then this can support the climatic case. Beside evidence from glacial movements, the record supplied by pollen maps is becoming increasingly valuable, especially in the form presented by T. Webb (University of Providence, Rhode Island) who has developed transfer functions based upon modern pollen studies which allow him to translate fossil pollen maps into projected climatic ones. A similar piece of work hs been conducted by N.A. Khotinsky (Moscow University) who has constructed a series of Holocene vegetation maps for the USSR, based upon fossil pollen data, which permit palaeoclimatic interpretation. Data emerging from this work may have profound implications, as, for example, the discovery of the elm decline at about 5,000 BP far beyond the supposed geographic limits of Neolithic cultures in the USSR. The case for climate (or disease?) is strengthened.

Since synchroneity is the key to these arguments, dating of deposits relating to palaeohydrological and climatic events becomes critical. This problem is particularly well demonstrated by the river channel studies of S. Kozarski (University of Poznan) in the Warta valley of central Poland. Aerial photography and geomorphological mapping have revealed the scars left by the river during the course of its development. In one location to the south of Poznan, the river once bifurcated and the valley which no longer carries the Warta River has the scars associated with a braided pattern. The main river valley shows two series of palaeomeander scars, an outer series with a mean curvature radius of 200 m and an inner series with a mean radius of 123 m. These old channels have become blocked and form oxbow lakes filled with sand and peat as swamp and fen vegetation has colonized and ultimately obscured them. The sediments at the base of the channels, however, provide evidence which is useful in determining their date of origin and hence aids the reconstruction of the history

of the river's development. Palaeobotanical evidence (from macroremains, such as fruits and seeds, and from pollen) is also helpful and has been analysed by K. Tobolski (University of Poznan).

The outer, larger series of palaeomeanders contains botanical evidence of an origin during the closing phases of the last glaciation. The pollen spectra are herb dominated and the major tree components are birch and pine. The dwarf birch (Betula nana), juniper, Artemisia and Chenopodiaceae all provide evidence suggestive of open, park-tundra vegetation typifying late-glacial conditions. Precise location of the sediments within the lateglacial chronology is difficult, on the palaeobotanical evidence, and one wonders whether the argument about whether the basal deposits date from before or after the Bølling Interstadial can ever be resolved on this basis. The observed changes in sediment and pollen stratigraphy could so easily be a consequence of inwash from the steep sides of the channels, thus confusing the stratification of the deposits.

Radiocarbon dating has been attempted and figures in excess of 11,000 BP have been obtained for certain levels, but the sediments are calcareous, making the precise interpretation of such dates difficult. It is reasonable, however, to conclude that the development of the wider series of meanders (which replaced the braided system) occurred during the lateglacial. This could be accounted for by the reduced flow resulting from the completion of ice wastage, the development of a complete (and partly wooded) vegetation cover, and the possibility of reduced precipitation.

The inner series of meanders proved to be considerably younger. The pollen spectra of the basal sediments within these are tree dominated, with oak predominant. Available radio-carbon dates suggest that some of these younger palaeomeanders began forming around 4,000 years ago, and some later than this. The smaller radii suggest that more water was moving down the Warta, but whether this was a consequence of climatic changes or human deforestation is impossible to ascertain.

The enigma of man's role in the palaeohydrology of central Poland is perhaps epitomized by the remarkable excavations of an Iron Age fortified settlement at Biskupin near Bydgoszcz. Built upon a low-lying peninsula extending into a 100 hectare lake this timber-built settlement, dating from 550 BC, has been extraordinarily well preserved as a consequence of raised lake levels swamping the wooden foundations. 35,000 stakes of oak and pine formed a palisade around the camp, which covered an area of about 2 hectares and within which were rows of wooden buildings, over 100 in all. The population was largely agricultural, growing wheat, barley, millet, beans, lentils and flax, and this arable emphasis, coupled with the timber necessary to build such a construction, must have involved extensive deforestation of the surrounding region. Which brings one back to the question of whether such forest clearance could have affected the hydrological regime in such a way as to lead to raised lake levels and the ultimate swamping of the settlement which finally led to its abandonment. Since this particular period in prehistory is generally believed to have been a time of increasing precipitation and lowered temperatures in northern Europe, this may have to remain just another palaeohydrological conundrum.

#### A particularly anomalous Seyfert galaxy

from J.H. Krolik

SEYFERT GALAXIES and their quasar cousins have provided much observational mash for the vats of theoretical speculation over the years since their discovery. A recent article by T. Heckman and B. Balick has thrown an especially juicy bucketful into this already potent brew. Ordinarily, what astronomers find extraordinary in these objects are the very large continuum luminosity (10<sup>43</sup>-10<sup>45</sup> erg s<sup>-1</sup> in Seyferts,  $10^{45}$  –  $10^{47}$  erg s<sup>-1</sup> in quasars) and the very strong, broad (103-104 km s<sup>-1</sup>) emission lines coming from an optically unresolved source believed to be no more than a few parsecs in size. In this new paper, attention is turned to extraordinary goings-on outside the nucleus of a Seyfert galaxy.

Heckman and Balick made vidicon pictures of the galaxy Markarian 335 with a series of interference filters covering bands centred on Ha and [O 111]λ5007, each 3,300 km s<sup>-1</sup> wide (FWHM), and extending in total over a range ±5,000 km s<sup>-1</sup> from line centre. Most of the line emission does indeed come from the galactic nucleus, but surprisingly, there is a significant flux in most of the observed wavelength range from regions clearly separate from the nucleus. Interpreting the emission as coming from a large photoionized nebula, the inferred mass of gas outside the nucleus is  $\sim 2 \times 10^8 \, n_{\rm c}^{-1} \, M_{\odot}$  and its kinetic energy  $\sim 2 \times 10^{58} n_e^{-1}$  erg, where  $n_e$  is its electron density in cm<sup>-3</sup>. Such a large mass of gas moving at such high speed has never been seen elsewhere.

The first question that comes to mind is the source of ionization to drive the Hα recombination radiation. Heckman and Balick check that it is plausible for the Seyfert nucleus to be supplying enough ionizing photons. Baldwin and colleagues2 previously showed that the nucleus of 3C 120 superionizes its H II regions. Here the Seyfert nucleus has a much more dramatic influence on its galactic environment.

The second question is the source of the gas. An obvious supposition, and one that Heckman and Balick consider, is that this gas is material that once radiated the nuclear broad emission lines but has now spread further out into the galaxy. Unfortunately, we have no clear way of telling whether the nuclear emission line clouds travel radially in, radially out, or go in any other direction. Some lines (for example, C (v) 1549) have profiles which are easy to measure, but are emitted isotropically, and so tell us nothing about cloud kinematics. On the other hand, those lines, such as La, which are emitted only from one face of a cloud<sup>3</sup> are often so distorted by blending or intervening absorption that their usefulness is destroyed. Therefore, the presence of this gas may be as good an indication that at least some Seyfert nuclei expel gas some of the time as asymmetry of nuclear emission line profiles.

Once the possibility of a nuclear origin for this gas is admitted, many other questions instantly arise. Why did the expulsion last only for 106 years (its kinematic age)? Seyfert galaxies are generally supposed to live at least 108 years. Why did it occur only in Markarian 335, a galaxy which doesn't appear remarkable in any other way (but one, which we discuss in the following paragraph). In their kiloparsecs-long passage out into the galaxy, why haven't the clouds been appreciably slowed by drag? Why is the shape of the nebula so strongly asymmetric?

The final, and perhaps most interesting, question stems from Heckman and Balick's measurement of the radial profile of the galaxy's continuum emission. They find that the surface brightness declines away from the centre in a way more characteristic of elliptical galaxies than spirals. If Markarian 335 is an elliptical galaxy, it would be the only such Seyfert galaxy known; all other Seyfert galaxies whose types have been determined are spirals. Ordinarily, when an elliptical galaxy has a line-emitting nucleus, it is also a bright radio source, which Markarian 335 is certainly not. On the other hand, Seyfert galaxies sometimes do evince small-scale radio emission near their cores4. VLA observations of this unusual Seyfert galaxy might be a particulary fruitful next set.

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#### The 1980 geomagnetic reference field

from David R. Barraclough

GLOBAL descriptions of the geomagnetic field are useful for many scientific purposes as well as being important in navigation. Traditionally, the information has been displayed by means of charts showing isolines of the geomagnetic element in question. Whilst this is adequate and even desirable for many navigational purposes, it has disadvantages for scientific use. During the last quarter of a century mathematical models of the geomagnetic field have been produced in large numbers to meet the needs of scientists and modern World Magnetic Charts are themselves

produced using such models as the first stage in the compilation process.

In almost all cases, the modelling technique used is the expansion of the geomagnetic field in terms of spherical harmonics. In this method, pioneered by Gauss almost 150 years ago, the potential (V) from which the geomagnetic field is derivable is expressed (equation (1))as

$$V = \alpha \sum_{n=1}^{N} \frac{n}{(\alpha/r)^{n+1}} \sum_{m=0}^{n} (g_n^m \cos m\phi + h_n^m \sin m\phi) P_n^m(\theta)$$

Here,  $\alpha$  denotes the radius of a reference sphere, usually taken to be the mean radius

(6,371.2 km) of the Earth, r the geocentric radial distance,  $\theta$  the geocentric colatitude and  $\phi$  the longitude of the point at which the potential is to be found and  $P_n^m$  ( $\theta$ ) denotes the associated Legendre function of degree n and order m. For a complete description of the Earth's magnetic field the series in equation (1) should extend to  $m=n=\infty$ , but in practice the series is truncated at a maximum value (N) of m and n, usually in the range n to n the geomagnetic field and its components can then be derived by taking the gradient of n as given by equation (1). A spherical

					icai narmon	ic coefficient	s of the inte	rnatio	nai	geom	agnetic referer	nce field 198	SU		
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_	3	1	-404	-366	-333	-335	0.2	g	8	4	0	-3	-8	4 7	0.0
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		5	-97 -62	-91	-83	-78	1.3	h	9	6	10	10	10	9	
g : h 5		5	-62 81	-56 83	-49	-48	1.4	g	9	7	5	. 3	4	7	
		0	45	43	88 45	92 49	2.1	h	9	7	10	11	11	10	
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g 6	,	6	-111	-112	-111	~108	-0.1	g	10	4	-2	- 1	2	- 2	
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g 7		1	-57	-57	-56	59	-0.8	h	10	5	-4	-4	~4	4	
7		1	-61	~ 70	-77	-83	-0.4	g			4	4	4	3	
7		2	4	1	_1	2	0.4	h			0	0	- 1	- 1	
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g 7		3	13	14	16	20	0.5	h			-2	1	1	-2	
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g 7		4	-26	- 22	-14	-13	1.6	h			3	3	3	4	
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harmonic model of the geomagnetic field thus consists of the N(N+2) coefficients  $g_n^m$ ,  $h_n^m$   $(m=0, 1, 2, \ldots, n; n=1, 2, \ldots, N)$  in equation (1).

Because of the relatively low truncation levels used, such models represent the parts of the geomagnetic field with wavelengths upwards of several thousand kilometres, that is, that part of the field which originates, in the main, from sources in the Earth's fluid core. This so-called main field is important in ionospheric and magnetospheric studies, in cosmic-ray physics and in studies of magnetic anomalies caused by crustal rocks. Magnetic anomalies can give valuable information about mineral resources but it is necessary to remove from the observations a realistic regional field so that the shorter wavelength crustal structure can be revealed. In the past, such regional fields were usually derived in an ad hoc way for each individual area of interest and this gave rise to problems when anomaly maps of adjacent areas were combined.

As an aid to users, and in particular to those interested in the study of magnetic anomalies, the International Association of Geomagnetism and Aeronomy (IAGA) adopted, in 1968, an International Geomagnetic Reference Field (IGRF) describing the main geomagnetic field at 1965 by means of 80 spherical harmonic coefficients (N = 8). Since the field changes with time, an additional set of 80 coefficients describing the secular variation was also included to extend the period of usefulness of the model. By the early 1970s it was becoming obvious that inaccuracies in the secular variation coefficients were causing unacceptably large errors in field values computed for current epochs from the model and the IGRF was revised by IAGA in 1975. This revision was limited to the provision of a revised set of 80 secular variation coefficients to be used for deriving field values for dates after 1975, the original and revised versions of the IGRF being continuous at 1975. With the passage of time this revised model has itself become inaccurate and a second revision of the IGRF was made at the IAGA Assembly held in Edinburgh last August.

The new version of the IGRF consists of five component models: four models of the main geomagnetic field for 1965, 1970, 1975 and 1980 and a model of the secular variation valid for the interval 1980 to 1985. The four main-field models each consist of 120 coefficients (N=10) whilst the secular variation model has, as before, 80 coefficients. The values of the coefficients are given in the Table. The constituent models have been derived by taking weighted means of three sets of candidate models, one each from the Institute of Geological Sciences in the UK, NASA (Goddard Space Flight Center) in the USA and the United States Geological Survey. The main-field models for 1965.

1970 and 1975 are designated Definitive International Geomagnetic Reference Fields (DGRF 1965, DGRF 1970 and DGRF 1975, respectively) since further revision is not envisaged. Linear interpolation between neighbouring models is to be used for dates that lie between the epochs of the models. The main-field model for 1980 and the secular variation model for 1980-85, which together constitute IGRF 1980, will be revised at a later date, probably about 1985. For dates between 1975 and 1980 a Provisional International Geomagnetic Reference Field (PGRF 1975) is defined by linear interpolation between DGRF 1975 and the main-field coefficients of IGRF 1980. For the interval 1980 to 1985, the main-field and secular variation coefficients of IGRF 1980 are to be used, according to the expression

C(t) = C(1980) + C(t-1980) where t denotes the date, C a main-field coefficient and C the corresponding secular variation coefficient. All coefficients are given in the Schmidt quasinormalized form and refer to a sphere of radius 6,371.2 km. For conversion from

geodetic to geocentric coordinates the use of the International Ellipsoid is recommended. This ellipsoid has an equatorial radius of 6378.160 km and a flattening factor of 1/298.25.

Computer programs for synthesizing field values and coefficients of the IGRF in computer-readable form are available from the following data centres: World Digital Data Centre Cl, Geomagnetism Unit, Institute of Geological Sciences, Murchison House, West Mains Road, Edinburgh EH9 2LA, UK; World Data Center A, National Oceanic and Atmospheric Administration, EDIS/NGSDC (D62), 325 Broadway, Boulder, CO 80303, USA; World Data Center A for Rockets and Satellites, Code 501, NASA/Goddard Space Flight Center, Greenbelt, MD 20771, USA.

The evaluation of the candidate models and the specification of the revised IGRF were the responsibility of Working Group I-1 (Chairman, N.W. Peddie) of IAGA.

David R. Barraclough is Co-chairman of the IAGA Working Group I-1 at the Geomagnetism Unit of the Institute of Geological Sciences, Edinburgh.

# Meteoritics — more facts, more complexity

from R. Hutchison and C.T. Pillinger

A recent meeting of the Meteoritical Society\* opened with a commemorative address for Harold Clayton Urey who died earlier this year, delivered by one of his former students, G.J. Wasserburg. The same speaker also gave the last lecture which looked into the future. His message, think isotopes and study only precisely characterized samples instead of random and heterogeneous bulk specimens, would undoubtedly have been approved of by his mentor. Meteorites are complicated objects (they seem to get more complicated all the time) recording chemical and physical processes which occurred before, during and after formation of the Solar System. They will only be divested of their information by much detailed research.

Between Wasserburg's opening and closing remarks, another former Urey student, Sam Epstein, reviewed data obtained by himself and F. Robert at Caltech concerning the deuterium/hydrogen ratios and other stable isotope compositions of the carbonaceous polymers of C1 and C2 chondrites which revealed that some fractions had several times more deuterium than terrestrial samples. This is an astounding result when compared to the parts per thousand

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Not to be outdone, N.J. McNaughton et al. (University of Cambridge) reported that for almost all the water extracted from bulk LL3 ordinary chondrites Bishunpur and Semarkona, the deuterium content was at least as high as that from the carbonaceous meteorites. Starting with samples like these, Urey might have made his Nobel Prize-winning discovery of deuterium a little earlier. The consensus of opinion was that deuterium enrichments are the result of ion-molecule reactions occurring in dark interstellar clouds and that a component from this source has survived within the Solar System as a heterogeneity in primitive meteorites.

Clearly, the deuterium story needs some of that painstaking effort demanded by Wasserburg. An example worth emulating is that of Ne-E, the almost pure <sup>22</sup>Ne, thought to be the daughter of short-lived <sup>22</sup>Na, which was first discovered in 1969. Thanks to the efforts of the Eberhardt group at Berne, who performed X-ray powder diffraction studies and semi-quantitative analysis during scanning electron microscopy, another of the host phases of Ne-E seems now to have been identified as a hydroxy or fluorapatite.

\*The 44th meeting of the Meteoritical Society was held at Berne, Switzerland, August 17–22, 1981.

Thus the parent <sup>22</sup>Na presumably was located in spinel, in a carbonaceous substance<sup>2</sup> and in apatite — the reasons for these diverse associations are not yet forthcoming.

Whilst the existence of a material which predates the Solar System is appearing more and more likely from Ne-E studies, the possibility that the Allende meteorite contains inclusions with ages up to 5,200 Myr — half a billion years older than the Solar System — appears to be receding. Employing the simple but elegant method of measuring the gas after irradiation, in a sealed ampoule, J.C. Huneke and I.M. Villa (Caltech) succeeded in showing that exceptionally high losses of <sup>39</sup>Ar (up to 65 per cent) can occur from fine grained Allende inclusions, probably by diffusion after recoil out of the fine grains into the grain boundary voids. E. Jessberger (Max-Planck-Institut für Kemphysik, Heidelberg), speaking on behalf of the group which reported the 'aged' Allende inclusions3, stated that appropriate repeat measurements would be performed with the added precaution of shielding from thermal neutrons in case these have some detrimental effect.

Changing views on the origin of Ca-Al rich inclusions in Allende and other carbonaceous chondrites were made clear by the reception given to G. Kurat's invited lecture. Eight or ten years ago, an origin by condensation was almost totally accepted for high temperature inclusions. Kurat (University of Vienna) was one of the few dissenters. Now, origins as melts, condensates and residues from evaporation are all considered, depending on the relevant chemical and textural characteristics of each.

There was much debate on the validity and refinement of estimates of cooling rates of meteorites based on different techniques4. Such information may be useful in determining minimum sizes for the parent planets of meteorites. K.H. Esbensen and V.F. Buchwald (Technical University of Denmark) showed that a 20 ton iron of the Cape York shower had formed by fractional crystallization from an Fe-Ni-S-P liquid down to about 700°C. Moreover, some of the products had not been significantly altered by solid state diffusion during subsequent cooling. Considerable chemical variation among the Cape York shower was described (D.J. Malvin and colleagues, UCLA), arguing against formation by simple fractional crystallization whilst part of the small planetary core thought to have been parental to all irons of group IIIAB.

R. Hutchison et al. (British Museum) defended their hypothesis<sup>5</sup> of hot accretion and rapid cooling for the formation of H-group chondrites, one aspect of which was questioned by J. Willis and J.I. Goldstein<sup>6</sup> (Lehigh University). A discrepancy between the metallographic cooling-rate (0.5°C Myr-1) and the Pufission cooling rate (> 3.8°C Myr<sup>-1</sup>) of the Marjalahti pallasite was announced by P. Pellas and colleagues (Museum d'Histoire Naturelle, Paris). If the latter is confirmed it would remove the inconsistency of the planetary core (IIIAB irons) seeming to have cooled faster than the core/mantle interface (main group pallasites) which is its outer boundary.

The importance of recent Antarctic meteorite finds provided a recurrent theme of the meeting. Collections of samples from areas of blue ice on the Antarctic continent by US and Japanese expeditions seemed to be offering meteoriticists guaranteed employment for some time to come. As detailed studies have got under way, however, it transpires, not unexpectedly, that many samples are paired and are thus representative of meteorites which fragmented7. Both S.G. McKinley (Albuquerque) and M. Honda (Tokyo) provided invaluable pairing information which should save colleagues unnecessary duplication.

The recovery and mechanisms of concentration of Antarctic meteorites were discussed by L. Schultz et al. (Mainz) and J. Annexstad (Johnson Space Center, Houston). One factor is wind, which can blow masses of up to 20 g across bare ice, to be concentrated at the snow covered margin. Earlier hopes of obtaining a supply unweathered meteorites from Antarctica have been dashed over the past few years. An example was presented of a small, dark stone sitting in a pool of water. although the air temperature was -20°C! However, as long as samples are unweathered, even though they may have spent a lengthy period on the ice cap before recovery, they may be considered as uncontaminated in terms of trace elements, like museum specimens observed to fall and immediately collected and carefully preserved (M.E. Lipshutz, Purdue).

Another facet of the paper by Honda was that the terrestrial ages of Yamato meteorites range from  $10^4$  to  $7 \times 10^5$  years, the mean life of an Antarctic meteorite probably being about  $2 \times 10^5$  years. Our sample of ancient meteorites from Antarctica is essentially the same as that from the rest of the world.

Several rare or unique meteorites have been recovered. H.Y. McSween and A.M. Reid (University of Cape Town) described the first observation in a meteorite of a contact between two primary, igneous lithologies, which probably formed in a single magma chamber. From the other side of Antarctica came a 189 g meteorite intermediate in composition between diogenites and eucrites (H. Takeda and H. Mori, Tokyo). Finally McKinley and colleagues (University of New Mexico) described a 'unique' unequilibrated, L3, chondrite from Allan Hills. The stone is unusual in that it contains both magnetitegraphite and silicates in the matrix, and so is intermediate between 'normal' unequilibrated ordinary chondrites and a recently discovered graphite-magnetite rich variety8.



#### 99 years ago

The Japan Gazette of August 21 contains a long and curious description of a bear festival among the Ainos. The writer, Dr. B. Scheube, is, we believe, the only European who has ever been actually present at this ceremony, the descriptions of it given by Miss Bird and other writers being derived from hearsay. The festival is now rarely held, and there is small reason to regret this, as it has degenerated to a brutal orgy. It commences with drink, every change in ceremony begins and concludes with drink, until finally every one in the village is intoxicated, while their hands, faces, and clothes are smeared with the gore of the sacrifice. Dr. Scheube says: "I had much difficulty in keeping off the drunken crowd that wanted me to partake of the blood and liver (the latter is eaten raw); and I can say that though hardened in these things by the practice of my profession, the sight of these drunken people with their bodies smeared over with blood filled me with a loathing that made me feel glad that the day and the feast were

coming to an end together". Dances, many of them of an obscene nature, also form part of

Mr. Stanley has published separately a full report of the address he recently gave in Paris. From this we glean one interesting item of exploration. After he had launched his steamer on the upper waters of the Congo, above the cataracts, he proceeded up the river and entered the Kwango, the great southern tributary. One hundred miles from its mouth he came to where two large streams united to form the main river; a greyish-white stream from south by east, the other, of an inky colour, from east by south. Ascending the latter, much less rapid than the former, Mr. Stanley came, after steaming another 120 miles, to a large lake, into which the river widened. On circumnavigating it, he found it about seventy miles in length, and with a breadth varying from six to thirty-eight miles. The natives he found very wild, and naturally astonished at the puffing monster. A splendid country the shore seemed to be impenetrable - lofty forests, alternating with undulating grass lands. Mr. Stanley was altogether three years away from Vivi, and doubtless he has collected much information in the country around the Congo.

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#### AUTUMN BOOKS SUPPLEMENT

## Who will write more books?

BOOKS have an odd place in the scientific literature. The institution of the book, especially when bound in hard covers, still commands general respect. Most books, after all, are the products of hard work and devotion by one or a few people. And not merely authors but attempted authors know how lonely their work is, and how it may give hostages to fortune. Yet some books have a lasting influence. Newton's *Principia* and Darwin's *Origin of Species* are but the most conspicuous if almost hackneyed examples.

Books are also, however, widely suspected. And with some reason. Although few books are now published entirely without the benefit of external criticism, peer review does not apply as formally as to articles published in the journals. Often, indeed, the authors of books appear to shoulder responsibility for external review themselves, thanking a small group of friends for help and advice immediately before the ritual disclaimer in most prefaces that only the author should be blamed for the errors that survive. And while some publishers may take great pains to ensure that manuscripts are read with care, and revised if necessary, the procedures of formal review are not uniform either between publishers or books, and are rarely in any case explicit. This is the sense in which books are often regarded as peripheral to the scientific literature proper.

Accordingly there is a temptation among professional people to suppose that books are somehow half-way between Apocrypha and light entertainment, in contrast to the measure of respect accorded to at least some journals. Books, then, are at a disadvantage in professional esteem compared with articles in the journals — a sad reversal of the traditional opinion that printed books are in some permanent way the embodiment of scholarship. So journals now command the attention of authors, who are reluctant to put more than a few thousand words on paper. Moreover, the scorn of books as vehicles of scholarship is probably greater among scientists than among other professional scholars.

It is high time that this balance was redressed. The importance of the journals in the scientific literature will not easily be undermined, and there is no reason why it should be, whatever the prospects ahead for electronic publishing and the like: but the value of printed books, especially those by a single author or by a few close collaborators is now too little appreciated. The result is that people's natural disinclination to suffer the pains of authorship for no immediate recognition is strengthened. The symposium of reviews of books that appears on the following pages may persuade some whose disenchantment is not permanently rooted that these neglected scholarly forms do indeed require more careful nourishment.

The most important need is that the contribution of books not merely to the education of students but of contemporaries should be more widely appreciated. Although most professional people are aware in their own education of the influence of particular books which seemed at first to be a means of coming to grips with some body of knowledge, and which later turned out to be a lasting challenge, it is now all too common that students, even undergraduates, are provided not with a book but with a list of references to articles in the journals (or even, illegally, with copies of them) and invited to make up their own books in their heads. By way of justification, it is often argued that this practice not merely teaches students about their subjects but that it also enables them to understand what research is like. This, however, is a thin excuse for many teachers' laziness.

A good textbook differs from the most carefully chosen set of

references in several obvious but nevertheless crucial ways. First, it will have a sense of history that cannot be conveyed by a list of dates. Second, it will judiciously assess the importance of contributions to some field of study. But it will also be challenging, stimulating and — ideally — original. Even in the most rapidly changing fields, good books for students have an element of reflectiveness of necessity absent from articles in journals. The appearance of Feynman's Lectures in Physics in the 1950s was a good illustration of how the very best textbooks become required reading for fellow-teachers as well as students. But, teachers will complain, good up-to-date textbooks simply do not exist in quickly moving fields. The remedy, they must know, is in their own hands, or pens.

Another common complaint is that there are too few scholarly reviews in the scientific literature. Over the years, especially in the United States, a succession of committees has considered how to encourage working scientists to withdraw for a time from the bench or the competition for space in the ordinary journals to distil their knowledge of a field or topic into a reflective review. Several remedies have been suggested. Review journals might help. So might fees for authors. In some fields these policies have worked well, sometimes commercially - without the need of subsidy. The results are widely appreciated, not only by people reading their way into a subject but by the scientific community as a whole. Yet the argument that there should be more reviews, and that scientists should be more willing to produce them, is only a part of the much stronger argument that there is an urgent need of more books, and that they too are a charge on people's professional responsibility. For books have the advantage over journals that they are — at least soon after publication — more accessible, more portable and more memorable. But who except professional scientists can meet the need to which they so often draw attention?

These didactic goals are the bread and butter of scholarship. But are the monumental books differently conceived? Not necessarily. Newton's *Principia*, indeed, reads as if it were a textbook, with its lemmas, theorems and the like; it was, after all, intended to instruct. And it is easy to see how the *Origin of Species* might have begun as a scholarly review of what was known, in the mid-nineteenth century, of the relationships between species. But in the end the result was a thesis. The moral is that even quite pedantic exercises in authorship may turn out to help to change the world. That is part of the fun, now apparently much neglected.

For many people, however, the potential excitement of authorship is outweighed by what are often considered to be the dangers inseparable from books. For books lend themselves to reflectiveness and also, unfortunately, to discursiveness. Thus they tempt authors into subjective judgements of other people's work, even to speculation. Is that not a temptation to resist, potential authors ask themselves? And is it not in any case dangerous to be published in a format which, it is well-known, includes much unfounded speculation hung loosely on a mass of unrefereed data, unestablished assumption and tendentious argument? The implied criticisms are unfortunately occasionally applicable. The conclusion is not, however, that all books are bad but merely that some are bad. Others, everybody knows, are at the other end of the spectrum. And their absence would impoverish the scientific literature as a whole. Is it not therefore time that the scientific community stopped complaining about the scientific literature and instead set about adding to the quality of that part of it which has been most neglected in the past few decades?

## The making of a modern museum

#### William Coleman

THE British Museum was largely the product of a single dazzling collection and the intention of an indefatigable collector, Sir Hans Sloane. Soon, however, that collection and the Will that transferred it to the British nation faced unexpected and, hindsight makes clear, increasingly unwelcome competition. Sloane had primarily collected natural objects plants, animals and minerals - and an associated library. Late in life he proposed that these materials, suitably housed and cared for, should form a repository in which his fellow countrymen could, as Robert Hooke had earlier declared of the Royal Society's museum, "read the book of nature" itself. But conflict was inherent in the very act of creation of the British Museum (1753). Sloane's treasures were confounded with two additional and equally remarkable collections: The Cottonian library and the Harleian MSS. Here were books and documents, important artefacts of human culture, that became and remained formidable rivals of the artefacts of nature.

Until 1880 these varied materials and numerous others, particularly archaeological objects and monuments of early European civilization, slept together in crowded confines in Bloomsbury. Their coexistence pleased few. Antonio Pazzini, Principal Librarian (that is, senior executive officer), was an imperious administrator and the impassioned developer of the Museum's printed materials and manuscripts, the heart of today's British Library. Until 1856 the scientific collections possessed no comparable advocate. From that date, Richard Owen, anatomist, zoologist and skilful political manipulator, directed a course that led to the opening, on 18 April 1881, of Alfred Waterhouse's visually superb new home for the scientific collections. The move to South Kensington in 1880-1883 thus provided physical autonomy for the natural history collections; complete independence (except for its cumbersome name) came only in 1963 when Sloane's original foundation was divided and the British Museum (Natural History) acquired its own board of trustees, including a majority of scientists.

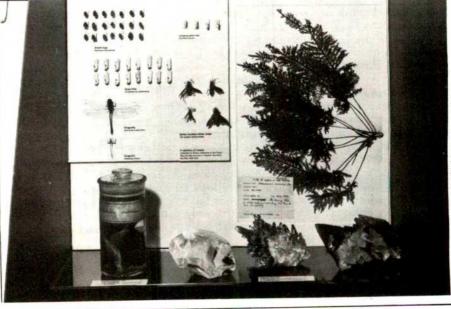
W.T. Stearn and A.E. Gunther, in dissimilar but complementary books, record in abundant detail the many and often complex developments and conflicts that produced today's British Museum (Natural History). Stearn's is a truly comprehensive

The Natural History Museum at South Kensington: A History of the British Museum (Natural History) 1753–1980. By William T. Stearn. Pp.496. ISBN 0-434-73600-7. (Heinemann: 1981.) £15. The Founders of Science at the British Museum, 1753–1900. By A.E. Gunther. Pp.219. ISBN 0-950-72760-1. (Halesworth, Suffolk, UK: 1980.) £9.90, \$20.

account, dealing with all aspects of the institution: Gunther explores, largely biographically, selected scientific work carried out by the staff at the Museum during its early years. Printed materials, official and unofficial reports and memoranda, correspondence and scientific publications provide the basis for these volumes. Gunther refers briefly and adequately to his sources but Stearn, alas, one of the foremost students of the bibliography of natural history, provides no overall guide to the marvellously diverse materials that he has consulted; his references lie scattered throughout the text and will need to be extracted by each reader as occasion dictates. Both volumes are well illustrated, the authors' selection of pictures affording a view of most features of the Museum's activities, including curation, exhibition and administration. Also valuable are the several appendices offered by each author, listing chronologically persons and major events associated with the Museum.

A museum, particularly a museum as important and well frequented (two to three million visitors per annum) as is the British Museum (Natural History), constitutes a diverse and expensive institution that inevitably must try to serve many masters. Its public face may appear orderly and calm but its private life is one of sharply differing opinions and compromise, the latter often offering satisfaction to few or none. Gunther and especially Stearn provide a record of the struggles, some personal and others substantive, that have constantly beset the Museum but neither author provides a sustained general consideration of the one issue that remains in question in all seasons, namely, what are and should be the principal functions of a (natural history) museum and how are these goals best to be realized.

Preservation and exhibition of specimens were intrinsic to Sloane's conception of a national museum; the further purpose of increasing the collections in size, and ultimately in systematic diversity, was added in later years. Only slowly did the British Museum (Natural History) come to view scientific research and technical advising as part of its concern. The example and the constant urging of John Edward Gray (1800-1875) encouraged this trend, one that required appointment of a competent scientific staff. But this was a matter for long years of no particular interest on the part of the Trustees. Such concerns continue, of course, and the demands that they pose are only heightened when limitations on staff,



urtesy of the Trustees of the British Museum (Natural History)





The public's view of the Natural History Museum in the 1980s. Opposite — part of the "Nature Stored: Nature Studied" exhibition, a display in a more traditional mould. Above left — the artefacts of nature remain, but push-button displays obtrude in the "Man's Place in Evolution" exhibition. What would Richard Owen (right) have made of it all?

funds and space become severe. The latter is a chronic museum problem and one often felt by the British Museum (Natural History). Confusion of function has been further compounded by the perceived tastes of the television generation: museum exhibitions and popular publications admittedly seek the lower end of a vague average intellect. They seek their market, attendance having become a crucial statistic with which to defend old support and to solicit new funds, by offering gentle instruction and mounting increasingly brilliant displays. Old values have in the process been transformed and the new product - the word fits well - is perplexing.

In this world agog with educational technology and associated display techniques, one must ask a foolishly obvious question - what use the artefact itself? It was the very object whose protection was the minimal original objective of any serious natural history collection, yet many modern displays, including the "spectaculars", offer more aluminium and plastic than rock, cellulose or flesh, however dry. Study collections, the hard core of a museum in confident times past and today, as extinctions proceed apace, one of our few hopes for further increasing our understanding of the career of life on this globe - occupy the back room; the public is given a glance at most. The moral is obvious: as display divorces itself from artefact, so might this circus in miniature easily and effectively separate itself from a central museum. Modern displays are instructive and entertaining but they are also reproducible; they can play in Palermo or Peoria as well as in London or New York. So perhaps they should, while the central museums redirect their limited resources to the care, study and display of the genuine objects of natural history.

William Coleman is Professor of History of Science and History of Medicine at the University of Wisconsin, Madison.

## The grand Vernonian omnibus

Robert Fox

Gentlemen of Science: Early Years of the British Association for the Advancement of Science. By Jack Morrell and Arnold Thackray. Pp. 592. ISBN 0-19-858163-7. (Oxford University Press: 1981.) £30, \$59.

THIS summer, in York, the British Association for the Advancement of Science celebrated its 150th anniversary. There could have been no more appropriate location. For it was in York, in 1831, that some 350 "friends of science" set in motion what a friendly contemporary called "the grand Vernonian omnibus". (The reference was to the Revd William Vernon Harcourt, the son of the Archbishop of York and the central figure in the task of foundation.) By 1844, when the fourteenth of its peripatetic annual meetings took place, once again in York, the BAAS had completed a circuit of the main traditional centres of British cultural life - Oxford, Cambridge, Edinburgh and Dublin (London, then as now, being excluded as a possible venue) - and of some of the bigger industrial cities. On the way it had given unprecedented visibility to the scientific enterprise and signally advanced the incipient sense of community among Britain's men of science. Oxonians and Cantabs, manufacturers and clergy, military men and doctors, peers and politicians, even some women, had become united in an organization whose seemingly easy commitment to the value-free universalism of science in reality carried a carefully constructed ideology.

Gentlemen of Science is a meticulous study of the first 13 years of the BAAS, in

which these successes were achieved. They were decisive years for science in Britain: the neologism "scientist" won acceptance, and more importantly, as Morrell and Thackray argue, the very idea of science as a distinct form of knowledge, at once benign, powerful and progressive, was articulated. In the process, Harcourt's Baconian conception of the Association as a vehicle for the predominantly empirical work of his fellow provincials gave way to a rival conception, emanating from London and Cambridge, which stressed the primacy of theory and mathematics. Inexorably, it seems, the intellectual weight of the Association drifted towards Section A (mathematical and physical science), and it was in mathematical physics that the early BAAS established its most distinctive and coherent tradition of research. Moreover, as mathematical physics rose in the BAAS hierarchy of the sciences, the biological and social sciences - agriculture, ethnology and geography, for example fell, while phrenology and education were among the would-be sciences that were edged out altogether. Even medicine succumbed as the Association's leaders took upon themselves the task of defining "science": it was no coincidence that the medical section disappeared in 1848.

The waters which the BAAS chose to sail were often choppy. Tractarians and fundamentalists joined the most conservative elements in political life in seeing the organization as a rival claimant to the various areas of authority which they regarded as their preserve. Then there was always the problem of competing interests within its

ranks. How, in particular, did it find room for two such abrasive and mutually hostile personalities as Sir David Brewster and William Whewell, the former virtually unemployed and obsessed with the inadequacy of scientific patronage, the latter basking in the ample luxury of Trinity College, Cambridge? One answer seems to lie in the prosopography of the twenty Gentlemen of Science whom Morrell and Thackray identify as the ruling coterie of the BAAS (and who give the book its title). All but two of the twenty were Anglicans of a liberal or latitudinarian persuasion, and most of them were also Whigs or Peelite Tories with an interest in moderate reform. They were, in short, predominantly men of the centre, wedded to an ideal of conciliation and social harmony.

It is one of the most impressive achievements of this impressive book to relate the spirit of accommodation which pervaded the higher reaches of the BAAS to the political and social conditions of the troubled decades that separated the massacre of Peterloo (1819) from the Chartist National Assembly of 1848. With mob violence a real or potential threat, and with established institutions being questioned as never before, there was every

reason for the gentry and aristocracy to feel beleaguered. They needed allies, and so reached down the social scale to make common cause with an increasingly powerful educated middle class which shared the aristocracy's horror of wholesale revolution. In this quest for social integration, science was quickly recognized as a natural field for shared endeavour, if only because it was deemed to be of such varied utility, whether as uncontroversial rational amusement, as the foundation of Britain's industrial prowess (a particularly dubious claim at the time) or as the source of at least a limited understanding of God. After two or three decades in which, for all these reasons, the vogue for science had gripped polite society, in metropolis and provinces alike, the conditions for the success of the BAAS could hardly have been more auspicious.

Gentlemen of Science is the fruit of more than ten years of painstaking research, in the course of which thousands of previously unknown letters (300 of them soon to be published in the Royal Historical Society's Camden series) have been culled to exciting effect. The authors bear their immense erudition gracefully, ingeniously mingling detailed narrative with passages

of analysis, and succeeding brilliantly (where so many others have failed) in elucidating the constant interplay between the content of scientific knowledge and the context of its production. The book bristles with challenging interpretations. If only by implication, it tilts effectively at the canard, launched by contemporary Jeremiahs like Charles Babbage and still alive to this day, according to which British science about 1830 was in decline. Babbage was, of course, correct in stating that science in Britain did not have the level of state patronage that it enjoyed in France. But what he did not say was that many French savants were in turn envious of Britain's tradition of self-help. That the French were justified in their envy is made abundantly clear by Morrell and Thackray's account of the BAAS's achievements, whether as a forum for the discussion and dissemination of ideas or as a lobby in what was at the time the new game of hunting research grants.

At last the early history of the BAAS seems to make sense. Above all, it is to be hoped that historians will never again try to squeeze the devotees of British science in the 1830s and 1840s into the uncomfortable strait-jacket of conventional models of professionalization. The aim of the Gentlemen of Science was not the advancement of science as a means of livelihood; they regarded the study of nature as a vocation whose interests were more likely to be harmed than served by the fetters of full-time scientific employment. Hence there was nothing paradoxical about their enthusiasm for colourful public displays, ranging from the sparkling dinner which the Archbishop of York offered at his palace during the 1831 meeting, to the impromptu sermon which Adam Sedgwick delivered "to some 3000 or 4000 colliers and rabble" on the beach at Tynemouth (Newcastle upon Tyne meeting, 1838). There was nothing paradoxical either about the fact that independent men of science like the Marquis of Northampton and the retired stockbroker Francis Baily occupied as prominent a place in the BAAS leadership as university chairholders like Sedgwick, Whewell and Baden Powell. By their work for the BAAS, these men undoubtedly contributed to the establishment of science as a profession. But they did so unwittingly. Had they been at York this year, they would have marvelled at our modern paraphernalia of formal qualifications and rigid career-patterns. They might also have reflected that science is no longer quite the fun which this absorbing and entertaining book shows it was 150 years ago.

#### **LERRILE**

GIVEN BY THE

#### Magistrates & Town Council of Glasgow LIST OF TOASTS.

- 5. The British Association & the Marquis of Breadalbane,6. The City of Glasgow, and the Lord Provost and
- America,

  9. The Memory of James Watt, and the other eminent men of Great Britain who have contributed to the
- 11. The Astronomers of the Continent, and Mr. Encke, .
  12. The Royal Society, and its Noble Chairman, the Mar-
- 12. The Royal Society, and its Noble Chairman, the Marquis of Northampton,
- The Foreigners who have contributed so much to the interest and success of this Meeting of the British Association,
- 14. The Ladies who have honoured the Meeting by attending its Sections,
- Railway Communication, & other Improvements which tend to facilitate intercourse between mankind, and thereby promote friendly relations,
- 17. The Rajah of Travancore, the great Promoter of Science in the East,
- The Commercial and Manufacturing interests of the Country, which owe so much to Science for their advancement,
- 19. Foreign Naturalists, and M. Agassiz,
- 20. The Lord Lieutenant of the County,21. The Secretaries of the British Association,
- 22. The Local Officers for the Glasgow Meeting of the
- Association,

CHAIR. Do.

Do. Do.

Do.

MARQUIS OF BREADALBANE.
LORD BELHAVENG

PRINCIPAL M'FARLAN.

GENERAL TSCHEFFEINE.

SIR JOHN ROBISON.
PROFESSOR AIREY.

MR. ENCKE.

MARQUIS OF NORTHAMPTON.

DR. BUCKLAND.

LORD SANDON. CROUPIER.

SIR D. BREWSTER.

LORD MONTRAGER.
MR. LYELL.

Ma. Phillips.

For the early members of the BA science was perhaps more funthan it is today, but must have had its tedious aspects. The illustration shows the list of toasts proposed at a dinner given for the Association by Glasgow Corporation in 1840.

Robert Fox is Reader in the History of Science at the University of Lancaster and President of the British Society for the History of Science. His most recent book, co-edited with George Weisz, is The Organization of Science and Technology in France, 1808-1914 (Cambridge University Press, 1980).

## In search of our place in the Universe

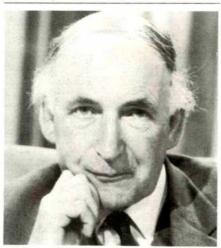
Joseph Silk

PHILOSOPHERS arise! Cosmology is beckoning. For mankind has found a niche in the Universe as it expands from an origin shrouded in mystery to an unknown future. Sir Bernard Lovell's new book tells the story of the development of human awareness of the Universe. Lovell leads us from the geocentric cosmology of the ancient world via the heliocentric cosmology of the Renaissance and the egocentric cosmology of the nineteenth century. His destination, of course, is the big bang theory of the expanding Universe, where fundamental issues still await resolution. Some of these issues differ little from the questions which faced the cosmologists of antiquity. Sir Bernard Lovell is an eminent radioastronomer who has no cosmological axe to grind, and it is especially refreshing to discover that his popular account of the evolution of cosmology manages to be both readable and enthralling.

We learn about the epicycles of Hipparchus and Ptolemy, and about how Thomas Aquinas incorporated Aristotle's crystal sphere cosmology into an apparently irrefutable argument for the existence of God. With Copernicus, the heliocentric hypothesis re-emerged and, against all odds, survived, while Tycho Brahe established the first accurate and systematic records of planetary data. Brahe punctured the immutable crystal spheres with his studies of comets and of the famous supernova of 1572. His assistant, Johannes Kepler, derived the laws of planetary motion after a painful decade of sifting endless mathematical permutations of the planetary data, and despite unflinching belief in the celestial harmony exhibited by the five regular solids. Next, Galileo's observations with a 4 cm aperture telescope caused an immense stir that led to an unavoidable conflict with the geocentric dogma upheld by theologians. Galileo saw sunspots, the moons of Jupiter and the phases of Venus, all of which established that the heliocentric system was a reality, and could no longer be thought of as a computationally convenient hypothesis. It remained for Isaac Newton to express the universal law of gravitation in a way that gave a theoretical understanding of Kepler's Laws.

The stage was set for modern astronomy. One of the great pioneers was William Herschel, who counted stars and mapped our Milky Way galaxy. Not, however, until the twentieth century was the Sun finally displaced from the centre of the Universe. Harlow Shapley resoundingly overthrew the egocentric view of the place of mankind in the Universe by using the newly calibrated period–luminosity relation for Cepheid variable stars to establish distances to remote globular star clusters. These were found to surround the

Emerging Cosmology. By Bernard Lovell. Pp.208. ISBN 0-231-05304-5. (Columbia University Press: 1981.) \$14.95, £10.80.



Sir Bernard Lovell, now tackling the history and philosophy of cosmology.

centre of our galaxy some 30,000 light years from the Sun. Much happened subsequently: the discoveries of the expansion of the Universe and of the cosmic microwave background radiation are two of the highlights that have now led cosmologists almost unanimously to adopt the big bang cosmological model.

All of this forms a backdrop to Lovell's theme, which is the evolution of cosmological thinking. Lovell's book forms part of a philosophical series entitled "Convergence" and founded, planned and edited by Ruth Nanda Anshen in order to explore the new consciousness which marks mankind's recently acquired ability to tinker with the evolutionary processes of nature. Our unfolding knowledge of the cosmos traditionally places cosmologists in the role of passive observers acquiescing in the thrill of new astronomical explorations of the frontiers of the Universe. Unlike most natural scientists, astronomers cannot perform controlled experiments in a warm laboratory; rather they take whatever the Universe has to offer.

One might think that such a discipline would have little in common with philosophers who concern themselves with the interaction between human beings and their environment. However a current cosmological theme provides a remarkable bridge between the philosophical undertones associated with the origin and fate of the Universe and the human perspective. This is the anthropic principle, according to which the properties of the Universe are determined by our presence as observers. Conditions must be congenial for intelligent life to evolve. This means, for example, that the early Universe could not have been highly irregular, inhomogeneous or chaotic, nor however could it have been perfectly regular, otherwise galaxies

and stars would not have formed. The anthropic principle accounts for the various large-number coincidences involving powers of 10<sup>40</sup>, the ratio of electromagnetic to gravitational coupling constants. These include the mass of a star and the mass of the observable Universe, as well as the coincidence between nuclear, stellar and Hubble expansion time-scales. It even purports to account for the approximate values of the constants of nature: if they differed significantly from observed values, human beings could not have evolved.

Where does this leave us? The uniqueness of our Universe can be attributed to our existence as observers, if we accept the anthropic principle. But have we really advanced our understanding of any aspects of cosmology? In 1925, A.N. Whitehead perceived about cosmology that "there is no parting from your own shadow". Is the Universe merely our shadow? Or perhaps the cosmologists are deceiving themselves and the roles are reversed. The truth may very well be that with only one Universe to explore, we can never resolve this paradox.

Joseph Silk is Professor of Astronomy at the University of California, Berkeley, and author of The Big Bang (W.H. Freeman, 1980).

## All around our star

David W. Hughes

The New Solar System. Edited by J. Kelly Beatty, Brian O'Leary and Andrew Chaikin. Pp.224. ISBN 0-521-23881-1. (Cambridge University Press: 1981.) £9.95, \$19.95. Orbiting the Sun: Planets and Satellites of the Solar System. By Fred L. Whipple. Pp.338. ISBN 0-674-64125-6. (Harvard University Press: 1981.) \$20, £14. Daytime Star: The Story of Our Sun. By Simon Mitton. Pp.191. ISBN US 0-684-16840-5; ISBN UK 0-571-11659-0. (Charles Scribner's Sons/Faber & Faber: 1981.) \$14.95, £10.

WE LIVE in the Solar System. Our planet, Earth, orbits the Sun and the theme of these three books seems to be that the more we know of such bodies the better. In effect we are presented with a progress report. Governments have spent large amounts of money on investigating our neighbouring planets and star. What have we got from it? Where do we go next? Why bother doing more? The books under review try to answer these questions.

The investigation of the Solar System has passed through three phases. The classical period encompassed the works of scientists such as Galileo, Copernicus, Kepler and Newton. This was a time of discovery and astronomy was almost entirely concerned with the planets, stars being

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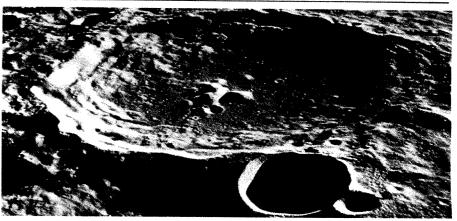
fixed points of light of little known significance. The second phase started in the late 1800s when astrophotography and spectroscopy spawned the discipline of astrophysics. During this period planetary investigation degenerated into a Cinderella science. The early 1960s saw the dawn of planetology. Since then spacecraft have been flung round, onto and into all of the planets known to the ancients, producing a veritable explosion of knowledge.

The first book under review is The New Solar System. Very rarely does one come across such an excellent account; I feel honoured to have a first edition as it is bound to run to many printings. The editors have collected together 20 chapters. each of about ten pages, written by a roll call of American scientists which reads like a Who's Who of US planetology. It is invidious to omit names, but as an indication of the standard it includes Eddy on the Sun, Chapman on asteroids, Burns on planetary rings, Wood on meteorites, Masursky on Mars, Shoemaker on the role of fragmentation and so on. The book concentrates on the comparative approach. It is much easier to discuss the surfaces of the terrestrial planets (Mercury, Venus, Earth and Mars) in one chapter and, for example, their atmospheres in another than to have an isolationist attitude to individual planets

The joy of reading the book is greatly enhanced by the illustrations. Not only does it bulge with space photographs, but it also contains a collection of coloured illustrations by artists Charles Wheeler, Don Davies and Jon Lomberg.

The editor's aim was to provide a book about the third phase of planetary exploration, the space-age planets, a book which would make enjoyable reading for those with either a professional or a casual interest. They have succeeded admirably. The fact that the publishers have produced this large format, beautifully illustrated, quality book at less than £10 is verging on the miraculous. I advise all readers to buy it

The second book is Fred Whipple's Orbiting the Sun: Planets and Satellites of the Solar System. This is a completely revised and updated edition of Whipple's classic text, Earth, Moon and Planets, which was first published in 1941. Whipple is Philips Professor of Astronomy, Emeritus, at Harvard University and Senior Scientist, Emeritus, at the Smithsonian Institution Astrophysical Observatory. He has devoted his life to the study of astronomy and space science, especially meteors, comets, space exploration planning, satellite tracking, novae and supernovae, Solar System evolution and practical astronomy; it is fascinating to read the view of such a man about the place of Earth and Moon within the planetary context. Whipple writes for the general reader and mathematics are avoided; however, the book does present the basic foundation of planetary astronomy and



The crater Daedalus on the far side of the Moon, photographed by Armstrong and Aldrin on July 21, 1969. The picture is taken from *The Atlas of the Universe* by Patrick Moore, a new edition of which has just been published by Mitchell Beazley, price £19.95.

does stress the underlying scientific principles. The author has also managed to integrate the three phases of Solar System investigation and we realize that all still have a role to play. We might move from bleary telescope images to the eye-opening, crisp detail of the space probe, but the Earth-based telescopes have recently revealed Pluto's moon, the rings of Uranus and Apollo asteroids. Also we realize that stars and planets are not to be compartmentalized. Whipple leaves us in no doubt that the origins and deaths of stars are intricately connected with the origin and evolution of our planetary system. The book is well written, well illustrated, well worth reading and a worthy successor to the previous editions.

My final book is *Daytime Star: the Story of Our Sun* by Simon Mitton. The word "Story" in the title describes the book well — Mitton dives into the subject and returns with a net bulging with intrigues. Why did ancient man worship the Sun and build stone circles to track its path? What drives the sunspots? Does the solar energy output vary slightly and thus trigger ice ages? What do neutrinos tell us about the solar interior? What have we learnt from space

about the solar atmosphere? Mitton has a racy style and we find atomic particles sloshing, neutrons bashing, gases toasting and gravity crunching.

There is much to be learnt about the Sun from this book, and it fills a definite gap in the astronomical library of the general reader. The author worried me when he stated that in 1964 "the finding of the solar wind was a result of astronomical observations of extremely remote radio sources" but 86 pages later he changes his mind and Biermann's 1951-1953 observations of comet tail phenomena are given the credit. I also found that the position of the Sun in the stellar hierarchy needed much more emphasis and that it is not enough just to say that the Sun has "an average size, typical mass, normal structure, temperature and luminosity". Granted the Sun is on the Main Sequence but it is also about nine times more massive than the average star and about 2,000 times more luminous. But these are minor quibbles about a book which is generally of a high standard and is definitely a good read.

David W. Hughes is a Lecturer in Astronomy and Physics at the University of Sheffield.

# The philosophy of astronomical discovery

David S. Evans

Cosmic Discovery: The Search, Scope, and Heritage of Astronomy. By Martin Harwit. Pp.334. ISBN UK 0-7108-0089-4; ISBN US 0-465-01428-3. (Harvester Press, Brighton/Basic Books, New York: 1981.) £12.95, \$25.

In the blurb Sir Fred Hoyle describes Cosmic Discovery as "A remarkable book. A unique book". So it is. It is an examination of the epistemology of astronomy, and one of those books which will set astronomers and philosophers thinking and talking. It is not a book which will command general acquiescence. Exactly how much influence it will have is hard to

say since it is certainly not everybody's cup of tea.

It starts off, oddly enough, with an idea connected with the collection of baseball cards. If one finds a duplicate, the set must be finite and if at any stage one counts up the number of single different cards (A) and the number of duplicates (B) the size of the set (n) may be estimated from the statistical formula n = A(A+2B)/2B. Harwit then applies this to astronomical discoveries of which he considers there are 43 distinct ones, and of these seven are recognized in two different ways. He is very careful about defining what he means by different ways, that is by totally unrelated

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techniques, perhaps best illustrated by the fact that the existence of galaxies was recognized by optical observations, but would now independently be found from radio observations. Putting A = 35, B = 7in 1979, with one triply recognized phenomenon (C=1), he estimates that there are 123 separate cosmic phenomena to be found of which we have now recognized 35 per cent. From a discussion of the rate of discovery he thinks we might know all of them by 2150 AD, a somewhat depressing conclusion for those who love observational astronomy, though favourable error bars would increase the numbers and postpone the date. Harwit distinguishes between original discoveries and their development, so that our successors may not all be unemployed by then.

He builds upon a figure published by Halton Arp in 1965 which defines the limitations of observations of extended visual sources considered in terms of their absolute magnitudes and linear diameters. In an elaborate analysis of techniques he considers the parameters of possible detection methods — frequency, size, spectral resolution, time resolution, ellipticity of polarization and intensity — and constructs multidimensional phase diagrams to show what is now accessible and what might become accessible in the future, and classifies each of his fundamental discoveries according to this scheme.

One chapter outlines his selected set of phenomena and the circumstances of their discovery. He makes a number of points in passing — for example that new discoveries follow the introduction of new techniques, with which few readers will quarrel. However he tends to think only of revolutions in techniques rather than in their availability. To an observer one of the most important events of the past few decades was the identification by Thackeray and Wesselink of both Cepheids and RR Lyrae stars in the Magellanic Clouds because of the newly available 74-inch reflector in the Southern Hemisphere. The resultant doubling of the scale of the Universe does not rank in his list of 43 discoveries. He mentions the serendipity of many discoveries, but fails to mention the outstanding discovery by John B. Irwin that S Normae was a member of the cluster NGC 6087. In fact his accounts of pulsating variables and flare stars leave a good deal to be desired and he fails to mention that pulsations continue down even into the white dwarf range. Indeed, one has the impression that in his accounts of fundamental discoveries Harwit confines himself to data from a rather restricted selection of well-known sources, especially those closest to the NASA publicity machine. For example, in his remarks on pulsars we fail to find any mention of the names of Cocke, Disney, Taylor, Nather or Warner, and the name of Lundmark is omitted from the story of the identification of the 1054 AD supernova remnant. The independent observation of

the Uranus rings by Joseph Churms gets no mention. Among other distinguished absentees, de Vaucouleurs rates no inclusion for the discovery of the local supergalaxy.

Though one can quarrel with Harwit's selection of phenomena and his historical accounts, perhaps this will not invalidate his main thesis for some readers. He remarks that many of the discoverers were not originally trained as astronomers, which is true but easily explained. The basic discoveries in radio, X-ray or gamma-ray astronomy were made before anybody could describe himself as an astronomer specializing in these fields. Until a few decades ago, employment opportunities in astronomy were so few that even aspirant astronomers took care to have an employable skill in some other field as a hedge against failure in the discipline of first choice. He remarks that by his reckoning the major proportion of recent discoveries has been made by Americans. though he might have added that even now a high proportion of top posts in American astronomy are held by individuals whose original training was received elsewhere, apparently himself included.

Lastly, he notes that many of the most original discoveries have been made by individuals outside the main stream of astronomical organization and funding. This, of course, has been remarked in other sciences; one can command organized research but it seems impossible to command true originality. However, insofar as observational astronomers in all fields are now married to large and expensive instruments, detailed planning and allocation of resources is essential.

The last part of the book contains Harwit's message - "How should we organize astronomy?". He argues for 13 recommendations, many of which are obvious enough - better training in physics and mid-career training in new techniques for astronomers, and vigorous introduction of exponents of new techniques in astronomy. Long-term grants will better allow astronomers to address fundamental problems and the current peer review system must be loosened up to encourage true innovation, "which can seldom be justified in advance". On the other hand, projects should not be kept on just because they have gone on for a long time. He wishes to encourage gravitational wave and neutrino astronomy and believes that in the electromagnetic domain current observing capabilities could be increased by a factor of 1,000 at modest cost. He finishes with a plea for policy to be set by panels of generalists of "unusual breadth of interest and far-ranging vision", more frequent policy reviews and frequent analyses of the factors which contribute to astronomical success.

These are fine words, but how to implement them in the real world with limited funds for pure research is another matter. In view of Harwit's desire to have more

frequent review committees, it is odd that he admits that "there is no evidence . . . that astronomical planning committees have in any way advanced the rate at which new astronomical phenomena are discovered . . . ". This is the paradox: astronomers need to spend a lot of money, much from public sources, and need to be accountable. They can guarantee the addition of large amounts of extremely valuable knowledge from their efforts. But the history of science demonstrates two things. One is that those who have predicted the exhaustion of the results of research have usually been proven wrong, however logical their arguments. The other is that discoveries of real originality are most often made by people who ignore the basic plan and pursue their own hunches. Many of them are rightly written off as cranks, but those who succeed are geniuses.

David S. Evans is a former Associate Director for Research at the McDonald Observatory and currently a Professor of Astronomy at the University of Texas at Austin.

## Relativity for all

D.J. Raine

Discovering Relativity for Yourself. By Sam Lilley. Pp.425. ISBN hbk 0-521-23038-1; ISBN pbk 0-521-29780-X. (Cambridge University Press: 1981.) Hbk £17.50, \$49.50; pbk £7.95, \$19.95.

ONE approach to physical science for the intelligent but innumerate layman is through analogy and example, omitting the why and wherefore. Sam Lilley has a different approach. He believes that students can be taught how to work things out for themselves.

Schopenhauer opined that mathematical truths properly presented can, in any case, be grasped intuitively, thereby circumventing the aridity of the Euclidean mode, for which he gave as sole justification a pictogram proof of Pythagoras's theorem. Lilley has a beautiful extension of this to the invariance of the interval in special relativity. But, even when only elementary mathematics is involved, not everything can be presented so easily; for example, on p.74 we must digress from space-time diagrams to explain negative numbers.

With general relativity the difficulties multiply. The equivalence principle is

The latest edition of *The Milky Way* by Bart and Priscilla Bok has just been published by Harvard University Press. The book, which first appeared in 1941 and has now been revised for the fifth time, costs £14.

discussed well in terms of accelerated observers in special relativity. But to master Lilley's ''near equations'' seems to require as much effort as would elementary calculus proper. Some limitations of the approach are apparent from the author's speculations on an alternative theory. This is incompatible with the precession of Mercury, but the reader (or author) is not provided with the confidence to do the relevant short calculation. Nor does one get any feeling for the results of the theory in cosmology, black holes or gravitational waves.

There are some mistakes: for example, a too-simple proof that bodies cannot move with the speed of light, and a peculiar notion of covariant tensors, which arises from confusing two meanings of "covariant". And amongst the more serious errors, two incompatible equations for the same gravitational force arise from an inconsistent approximation.

But for me the achievement outweighs these flaws of execution. Of the greatest importance is the demonstration, through a large number of well-made teaching points, that mathematics, the language of science, is available to anyone with sufficient motivation and the right guide.

D.J. Raine is a Lecturer in Theoretical Astrophysics at the University of Leicester. His books include Einstein and Relativity (Priory Press, 1975) and The Isotropic Universe (Adam Hilger, 1981).

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# From the shadows, everyman's Maxwell

R.V. Jones

James Clerk Maxwell: A Biography. By Ivan Tolstoy. Pp.194. ISBN 0-86241-010-X. (Canongate, Edinburgh: 1981.) £9.95.

FOR many years there hung in one of the corridors of the Electrical Laboratory at Oxford a photographic portrait of someone whom none of the staff could identify. Presumably it had been placed there by the Professor of Physics, J.S.E. Townsend; but after his death, the identity of the portrait was forgotten. It was, in fact, of James Clerk Maxwell.

Maxwell has been almost as little known in his native Scotland, where his memory has been heavily overshadowed by that of - and this despite the fact that Einstein himself credited Maxwell with having brought about the greatest revolution in physics since the time of Newton. Not only did Maxwell create the electromagnetic theory which led to the discovery of radio waves and of electromagnetic radiation pressure, but he also formulated his distribution law governing the molecular velocities in gases, the basis of automatic control theory and the principles of kinematic design; and he demonstrated the first colour photograph.

The current year has seen the one hundred and fiftieth anniversary of his birth on 13 June 1831, while less than two years ago there occurred the centenary of his death. The commemoration of these anniversaries has coincided with increasingly wide-spread recognition of his tremendous contributions, and with papers and books describing aspects of his life and work, notably C.W.F. Everitt's short but carefully written biography (Charles Scribner's Sons, 1975) and the exhibition of Maxwell memorabilia at last year's Royal Society conversazione.

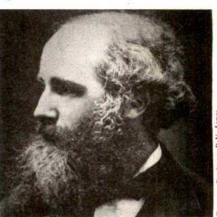
Now we are presented with another short and very readable biography, by Ivan Tolstoy, who in his preface sets out his objective:

For physicists the name of James Clerk Maxwell ranks next to Newton and Einstein. Yet among non-scientific people Maxwell's image is surprisingly faint. It is hoped the present book will help remedy that injustice. This pretends neither to be a definitive biography, nor a work of historical scholarship. It is, rather, a book for the lay reader.

Even so, and although the author has, as he says, drawn largely on secondary sources, more specialist readers will find that he has thrown shafts of light on Maxwell's character and methods of working. And in well-drawn quotations from Maxwell's own writings he has followed one of his subject's own precepts:

It is a great advantage to the student of any subject to read the original memoirs of that subject, for science is always most completely assimilated in the nascent state.

One of the rare points in the book which could be questioned concerns the process by which Maxwell came to conceive the famous displacement current in a vacuum. Tolstoy says that "There could be no physical or logical justification for keeping the concept in the context of a vacuum" This might be true if Maxwell regarded a vacuum as containing absolutely nothing, but in his 1864 paper he stated his belief in "an aethereal medium filling space" and in its having a "small but real density"; and he evidently retained this view throughout his work, for towards the end of the Treatise of 1873 he repeated "We must therefore regard the medium as having a finite density" (para.782). So Maxwell's train of thought was both physical and logical.



James Clerk Maxwell, the portrait that hung in the Electrical Laboratory at Oxford.

Tolstoy remarks elsewhere that "Both Einstein and Maxwell were stronger in physical intuition than in mathematics" which struck an immediate resonance in my memory with something Einstein said in conversation with F.A. Lindemann during his visit to Oxford in 1931. He was commenting on the popular description of himself as a mathematician: "I am not a mathematician. I am a physicist — if I had been a mathematician I could not have done what I have". But, of course, both Einstein and Maxwell had an impressive command of mathematics when they needed it, and Maxwell was outstandingly strong in geometrical reasoning.

Tolstoy's short account is both penetrating and sympathetic regarding Maxwell's personal relationships, of which it gives a warmly human picture; and it contains many thoughtful comments on Maxwell's physics and the context in which his advances were made. It is a book well worth reading.

R.V. Jones is Professor in the Department of Natural Philosophy at the University of Aberdeen, and author of The Complete Physicist: James Clerk Maxwell 1831–1879 (Yearbook of the Royal Society of Edinburgh, 5–23; 1980).

ourtesy Professor R.V. Jones

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## Where is everybody? Some new answers

Gerard K. O'Neill

Are We Alone? The Possibility of Extraterrestrial Civilizations. By Robert T. Rood and James S. Trefil. Pp.262. ISBN 0-684-16826-X. (Charles Scribner's Sons: 1981.) \$14.95.

Is the origin of life so extraordinarily improbable an event that it has occurred but once in the history of our galaxy, or do star-systems other than our own teem with intelligent life, able to communicate with us? Surely this question, raised almost 40 years ago by Enrico Fermi, is one of the most profound that we can imagine.

In an engaging and readable book, Rood and Trefil outline an emerging modern view of the answer. Their style is informal and unpretentious, and the level is suitable for interested non-scientists, but they offer tid-bits of detail for specialists. Given the provocative and necessarily speculative nature of their subject-matter, it seems a happy choice on their part, as well as an honest one, that they include a chapter listing their own divergences of opinion and the arguments made on either side of the issues.

They begin with the seminal paper, "Searching for Interstellar Communications", by Giuseppe Cocconi and Philip Morrison, which appeared in Nature in 1959 (184, 844-846). There the authors pointed out that sensitive radio receivers could detect signals from transmitters of moderate power over interstellar distances. Frank Drake, already thinking along similar lines, was further stimulated by Cocconi and Morrison's paper and carried out Project Ozma, using a radiotelescope to listen for possible artificial signals emitted from the nearby, solar-type Tau Ceti and Epsilon Eridani systems. Although no recognizably artificial interstellar signals were ever detected, then or in searches over the succeeding decades, there grew in the early 1960s something of a consensus among those scientists interested in the question. It was based on the assumptions they made of probabilities: that a star will have planets, that life will develop on one such planet, that it will develop technological expertise and that a culture capable of communication will survive long enough to be heard. Those probabilities and a few other factors, simply multiplied together, form what is variously called the Sagan-Drake, the Drake or the Green Bank Equation. Rood and Trefil choose the last version, as a memorial to the first scientific conference (1961) on interstellar communication. The participants at the Green Bank conference concluded that intelligent life was abundant in our galaxy, that physical space-travel over interstellar distances would be forever impractical and that communication could occur only by radio transmissions; the only problem was to find the right frequency and the signal code.

One definite signal, unmistakably intelligent and extraterrestrial, could end the argument at any time, but until that happens everyone can speculate. Most of Are We Alone? is devoted to the fascinating skein of logic woven over the past ten years by a younger generation of scientists, who have reached a fairly general agreement among themselves that the Green Bank participants were both too sanguine on their probabilities and too short-sighted on technical progress. In the modern view, life is a far more unlikely phenomenon than had once been thought, and there is a strong possibility that it is unique to our planet.

Michael Hart stimulated much of the new research on the Fermi question, and the authors discuss Hart's work in considerable detail. Dr Hart, now at Trinity College in Texas, set up a mathematical model of the Earth's atmosphere, and in 1978 traced the evolution of that atmosphere by computer over geological time, from its formation by volcano emissions to its complete transformation through biochemical processes once life had formed. Hart found that the survival and evolution of life depended on an extraordinarily lucky series of accidents, by which the temperature of the Earth remained remarkably constant for most of the age of our planet. Had we not been so fortunate as to be located at precisely the right distance from the Sun, within margins of no more than one or two per cent, Earth would now be as lifeless as Mars or Venus, according to Hart's calculations.

The young revolutionaries further conclude that their elders were badly

The opening lines of the seminal paper by Cocconi and Morrison (1959) which stimulated others to listen for signals from space.

No theories yet exist which enable a reliable estimate of the probabilities of (1) planet formation; (2) origin of life; (3) evolution of societies possessing advanced scientific capabilities. In the absence of such theories, our environment suggests that stars of the main sequence with a lifetime of many billions of years can possess planets, that of a small set of such planets two (Earth and very probably Mars) support life, that life on one such planet includes a society recently capable of considerable scientific investigation. The lifetime of such societies is not known; but it seems unwarranted to deny that among such societies some might maintain themselves for times very long compared to the time of human history, perhaps for times comparable with geological time. It follows, then, that near some star rather like the Sun there are civilizations with scientific interests and with technical possibilities much greater than those now available to us.

misled by (in my phrase) "temporal chauvinism", a lack of appreciation of the probable development of technology beyond our era. If there is a civilization more advanced than our own, it will be ahead not just by decades but by millenia - and with technology to match. The authors conclude that the modern concept of space colonies, not published until more than a decade after the Green Bank conference, logically confounds many of the assumptions made at the time. Space colonies, they estimate, are certainly feasible technically, do not require a particularly high level of technology, and have such extraordinarily high survival value for any civilization reaching its nuclear age that they will inevitably be developed by any long-lived civilization capable of interstellar radio communication. Given the certainty of space colonies, virtually every star-system is a hospitable target for colonization by any species, no matter what its point of origin or requirements of temperature, gravity and atmosphere.

The development of space colonies therefore means, in the opinion of the young scientists of the modern school, that physical interstellar travel, first by robot probes and then by living colonists, is a natural and not particularly difficult stage in the development of any intelligent civilization. That conclusion is made quantitative by computer simulations carried out by Eric M. Jones at the Los Alamos Scientific Laboratory. Dr Jones comes to a single conclusion, remarkably insensitive to assumptions about ship speeds, the gestation time for a civilization around a star before it spawns colonies to move on to other stars, and other variables. He finds that the spread of a civilization outward across the entire galaxy takes no more than at most a few thousandths of the galactic age — that is, the expansion is explosive. My own calculations confirm that conclusion, and suggest an even shorter expansion time, less than one tenthousandth of the galactic age. I argue that the outward movement of self-replicating robot probes is more certain than that of life, but Jones may well be right in his assertion that, in our own case, "The human migrants will not be far behind the probes".

In the view of the young revolutionaries, based on that chain of logic, if there had ever been an intelligent race prior to our own its descendants would be here already. And as we seem to have evolved locally, we are not they. Hence, there's a strong probability that we are in fact alone in our galaxy. It is thought-provoking that Enrico Fermi, by his famous question "Where is everybody?" appears to have arrived at the same conclusion 40 years earlier.

Gerard K. O'Neill is Professor of Physics at Princeton University and President of the Space Studies Institute. His new book 2081 (Simon and Schuster/Jonathan Cape; 1981) explores the impact of technology on the human prospect.

# Snow: reflections on physics in our time

H.B.G. Casimir

The Physicists: A Generation that Changed the World. By C.P. Snow. Pp.192. ISBN UK 0-333-3228-2; ISBN US 0-316-80221-2. (Macmillan, London/ Little, Brown: 1981.) £8.95, \$15.95.

ALTHOUGH physicists like to insist that they are anything but narrow-minded specialists, only few of us have been professionally successful outside the field of science. C.P. Snow was one such rara avis. After an honourable career at Cambridge as a research fellow and later as a tutor at Christ's College, he became famous as a novelist and, moreover, distinguished himself as a Civil Service Commissioner. In his well-known Rede Lecture of 1959 he stressed the gap between "Two Cultures" but he himself tried to bridge that gap — his novels have given many readers a better understanding of the attitudes and aspirations of academic scientists.

In the present book, the first draft of which was completed just before his death on 1 July 1980, Lord Snow returns to science. In eleven chapters he depicts in broad strokes the stupendous development of physics during our century, tells about the main contributors to that development, explains its decisive influence on human society and dwells on the moral responsibility of scientists. In addition there are three appendices: Snow's farsighted editorial in *Discovery* of September

1939; Einstein's letter to President Roosevelt; and Snow's speech on the moral un-neutrality of science, delivered in 1960 to the American Association for the Advancement of Science. The book is abundantly illustrated with well chosen photographs and diagrams.

Most physicists will agree with the general tenor and with the message of Snow's book. Also, as was to be expected, Snow writes clearly and fluently, and he often succeeds in characterizing a physicist in a few pregnant sentences, for instance Fermi on p.79:

He had been recognized very young as one of the physicists of the century, and the only one who could work on equal terms with the greatest in both theory and experiment. There had been no one like that for generations . . . . If Fermi had been born thirty years earlier, it was possible to imagine him discovering Rutherford's nucleus, and then proceeding to Bohr's theory of the atom. . . . As a professional scientist, not as a cosmic thinker such as Einstein or Bohr, he was one of the very greatest.

William Cooper tells us in his introduction that Snow intended to write mainly from memory and it is clear that the book is not based on careful historical studies. It is a personal narrative, but a personal narrative by a man like Snow is well worth reading. For a physicist it is stimulating to compare Snow's choice and evaluation — often based on close acquaintance with the persons and the

subjects discussed — with one's own views. But a general reader should realize that Snow's presentation is often incomplete and may lead to a one-sided or even distorted picture: it is an interesting but not entirely reliable guide.

There is, for instance, no indication of the important role of kinetic theory and statistical mechanics in the development of the atomic theory of matter; Boltzmann is not mentioned, Gibbs only as the creator of chemical thermodynamics. Einstein's work in this field passes unnoticed also. Yet it was Perrin's experimental confirmation of Einstein's predictions concerning Brownian motion that convinced many unbelievers of the real existence of atoms. Similarly, Einstein's theory of the specific heat of solids at low temperatures convinced many physicists of the reality of quantization. When writing about crystallography and X-rays, Snow mentions W.L. Bragg (Sir Lawrence) and Bernal, but fails to mention Bragg's father (Sir William) and von Laue. Wave mechanics is called the De Broglie-Schrödinger theory; that is fair, but note of the curious fact that both De Broglie and Schrödinger did not accept - or did so only with reluctance - Born's interpretation of the wave function as a probability amplitude should not have been omitted. Snow draws a lively picture of Feynman and sketches his work on quantum electrodynamics. Dyson is also mentioned, Schwinger and Tomonaga are

Sometimes the Cambridge (or more generally the British) point of view does not apply elsewhere. When Snow writes on p.42 "Until the Second World War there was little industrial support for physicists" this may have been true in England but in the United States, in Germany and also in my own country industry employed many physicists, some of them even Nobel Prize winners. Further, at Cambridge spectroscopy was considered to lie outside the main compass of physics. Elsewhere it was felt that Rutherford and his school were pioneering an as yet lonely track, whereas the main action was to be found in the unravelling of spectra, which indeed eventually led to the understanding of the periodic system, to the exclusion principle, to the notion of electronic spin and to the semi-quantitative vector model, thus paving the way for quantum mechanics. It was in the 1930s, after the birth of quantum mechanics, that nuclear physics burst into

The book contains a number of plain errors, which Snow, had he lived, would presumably have corrected, and which a careful editor ought to have noticed. Uhlenbeck (p.113) was not involved in the mission of Goudsmit; the Göttingen Nobel Prize winner James Franck is turned into Josef (p.117) and the index lists both James and the non-existent Josef; A.H. Compton was an American, not a British Nobel Laureate (p.120). It was not Gell-Mann-



Germany in 1933 — students and Nazis burning books by foreign and Jewish authors. The slogan reads "German students march against the un-German intellect". Such scenes led to the flight of Jewish physicists from Germany and eventually to the development of the atomic bomb in the United States.

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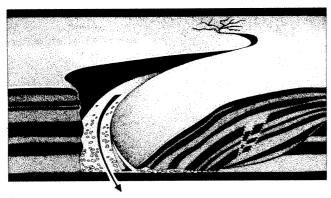
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1982, 370 figures. Approx. 700 pages Cloth DM 110,-; approx. US \$ 51.20 ISBN 3-540-11257-X

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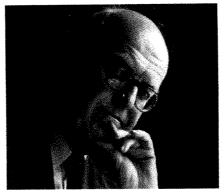
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who introduced group theory into physics (p.145): that had been done by Wigner in the late 1920s. Group theory then became one of the customary tools of theoretical physicists. Gell-Mann applied it ingeniously and successfully to the problems of particle physics.

In two cases Snow's tendency to sum up people in a few sentences leads to injustice. On p.112 he states bluntly that "General Groves was a singularly bad choice for his job". I have no well-founded personal opinion, but I notice that A.H. Compton writes in his Atomic Quest — by a curious coincidence also on p.112 — "The nation was fortunate indeed in the selection of General Groves for this task". Also, it cannot be denied that Groves's short-term mission was efficiently accomplished. The second case is worse. On p.26 Snow writes

... Lenard who, incidentally, as an old man was one of the only two eminent German scientists who became active spokesmen for the Nazi faith. (The other was Werner Heisenberg, a great theoretician...).

Such parenthetic calumny should not go uncorrected. Certainly, Heisenberg was a German patriot. He loved his country; the German language and German culture meant much to him (but in the early 1920s the *Jugendbewegung* to which he belonged was not a nationalistic group). We may regret that he let his love for his country prevail over his objections to the Nazis, we may wish that he had taken a firmer and more courageous stand against them. And I admit that on the one occasion I met him during the war he showed little



C.P. Snow 1905-1981

understanding for the feelings of people in an occupied country. But he always maintained his integrity as a physicist and at one time he was even under severe attack by members of the Lenard group, because he continued to express his belief in quantum theory and relativity. It is utterly wrong to mention him in one breath with Lenard.

Nevertheless, despite these detailed criticisms, I consider *The Physicists* a valuable book. Its theme is important, its basic structure sound and it contains many incisive remarks and interesting side-lights. I read it with pleasure and profit, and am convinced that many will do likewise.

H.B.G. Casimir studied theoretical physics at Leiden, Copenhagen and Zürich, worked in low temperature physics at Leiden and later joined the Philips Company at Eindhoven. He is a foreign member of the Royal Society and a former President of the European Physical Society.

# Philosophy in an uncertain world

John Polkinghorne

Divine and Contingent Order. By Thomas F. Torrance. Pp.162. ISBN 0-19-826658-8. (Oxford University Press: 1981.) £9.50, \$27.59

PROFESSOR Tom Torrance is a distinguished theologian, the chief apostle in this country of Karl Barth, whom many would regard as the most significant figure in twentieth-century theology. In addition, Torrance is one of the few British theologians to have taken a serious interest in what science has to say about the world. This reflects itself in Torrance's strongly objectivist approach to theology, and it is no accident that one of his best known books is called *Theological Science*.

His latest work is concerned with the assessment of the significance of the contingency that we find in nature. We can distinguish at least four senses in which such contingency is present: (i) The laws of nature are not necessary, in the sense that their form cannot be determined by pure thought alone but has to be obtained from experimental observation. The recognition and exploitation of this is what gave

modern science its superiority over that of the ancient world. (ii) The parameters appearing in those laws, such as the fine structure constant, take values which can only be determined empirically. Sir Arthur Eddington tried to deny this in Fundamental Theory and came to grief. However an interesting gloss on the values of these constants is provided by the anthropic principle which recognizes limitations to be satisfied in a universe in which we can emerge as observers. Torrance only mentions this in a footnote; it deserved greater consideration on his part. (iii) In the great diversity of things that happen there are unforeseeable concatenations of circumstance. This is the "chance" that Monod saw operating in the emergence of life and which for him (but not for me) caused the fabric of significance to crumble. (iv) There is the radical unpredictability of quantum mechanical observation and (I would say) the action of the will, both topics which we do not understand very well.

It seems important to discriminate between these four aspects of contingence,

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but I did not feel that Torrance always adequately observed the distinction.

As a matter of historical fact the Christian doctrine of creation helped men to recognize (i) and (ii) and so made possible the scientific revolution. By a curious twist, the Newtonian mechanical world view then tended to abolish the recognition of (iii) and (iv).

The presence of contingence in the world is combined with a high degree of intelligibility which enables us to understand it. Torrance says "The intelligibility of the universe provides science with its confidence but the contingence of the universe provides science with its challenge" (p.58). Such a view of the world is certainly consistent with the doctrine that it is the work of the sovereign will of the Creator and that its rationality is the reflection of his mind. Torrance seems to argue that only such a doctrine is possible but I cannot agree that there is that degree of intellectual compulsion. He sees creation as implying two complementary aspects. First, the world is wholly dependent on God since without his sustaining it would collapse back into nothingness. From this comes its contingent order. Second, the world is other than God (no pantheism!) so that he has made it to stand apart from him. The first aspect is the concern of theology; the second that of science whose method is to proceed etsi deus non daretur, as if God did not exist. A true understanding requires a synthesis of these aspects.

A test of any world view is its understanding of evil. Torrance equates evil with disorder but nevertheless (rightly I believe) does not go along with Augustine and Aguinas in seeing it as just the absence of good. I thought his discussion needed a more thorough-going eschatological dimension. Also, I did not always recognize the world of science as Torrance described it. He attributes a release from the shackles of Newtonian necessity to the creation of the field concept and to the genius of Einstein. I cannot see that. Partial differential equations have propagation properties as rigorous as those of ordinary differential equations and causality finds its place within the lightcone of relativistic physics. Further, I did not understand his attitude to mathematics. He does not like to accord it true intellectual independence (that would be dangerously Platonic and Plato's ideas are possible rivals to God in his eyes) so he says it has a "natural bond with nature". Again, I do not see that.

The book is written in a style which might be described as "Scottish professorial", a sort of intellectual cousin to the elaborate castellation of Scottish baronial. Certainly, it does not make for easy reading or rapid assimilation.

John Polkinghorne, formerly Professor of Mathematical Physics at Cambridge, is a Fellow of Trinity College and an Anglican clergyman.

# How not to make a splash in science

Robert Ubell

Polywater. By Felix Franks. Pp.208. ISBN 0-262-06073-6. (MIT Press: 1981.) \$15, £9.30.

In the early 1960s in an obscure laboratory in Kostroma, 190 miles from Moscow on the upper Volga, an equally obscure research scientist, Nikolai Fedyakin, stumbled on a surprising phenomenon. Looking at how liquids behave in very narrow capillaries, he watched as a dense new liquid formed in neighbouring empty tubes.

In Moscow, the renowned physical chemist B.V. Deryagin quickly recognized the possible implications of Fedyakin's 'discovery' and took it for his own. He set an entire laboratory to work on it, published results widely and campaigned for the recognition of anomalous water at Faraday Society and Gordon Research conferences. The new liquid was 15 times denser than normal water, boiled at temperatures much higher than 100°C and froze, without forming ordinary ice, at under -30°C. For a time, Western scientists either yawned or sneered. Some guessed it wasn't water at all, but the result of contamination. Yet excitement grew, and in England attempts were made to replicate the Russian work. J.D. Bernal, in private, called Deryagin's achievement "the most important physical-chemical discovery of this century".

The US Office of Naval Research jumped in next, sensing that the mysterious new form of water might have military uses. Ripples of interest swelled to waves: the liquid was given a name — polywater — and busy scientists suddenly found the time and money to work on what they had seen as a mere curiosity the previous week. Respected researchers and the overzealous alike scrambled to make their mark, even though, at most, only a few drops of polywater had ever been collected.

In 1973 the bubble burst. In a brief, dignified note in *Nature*, Deryagin reported that he and his colleagues had finally found it impossible to grow polywater from ordinary water. The unique qualities claimed earlier "should be attributed to impurities rather than to the existence of polymeric water molecules". The emperor was not well dressed.

Felix Franks escaped the perils of the polywater controversy, but as a distinguished surface chemist and an authority on water he was close enough to record it all. The result is this book. To his great credit, Franks treats those who believed and those who didn't with an even hand, praising little, blaming less. This is a skilfully made book, wise, urbane,

#### Nature of "Anomalous Water"

Many experiments have corroborated the phenomenon involving the formation of condensates with anomalous properties from the vapours of water and other liquids on silicate surfaces. But the nature of this phenomenon remained obscure for a long time and widely differing hypotheses were put forward to clarify it, one of them involving the formation of stable associates of water molecules (H<sub>2</sub>O)<sub>n</sub>. This hypothesis was first formulated by us<sup>1-4</sup> and was developed further by Lippincott *et al.*<sup>5,6</sup>.

We have established that there are no condensates both free of impurity atoms and simultaneously exhibiting anomalous properties. Consequently, these properties should be attributed to impurities rather than to the existence of polymeric water molecules.

Consequently, the anomalous properties of condensates may be explained, not by the formation of a new modification o water, as was previously supposed, but by the peculiar features of a reaction taking place between the vapour and solid surfaces in the process of condensation. Many aspects of the mechanism of formation of anomalous condensates have not yet been fully clarified. This especially applies to the formation of anomalous condensate on MgO surfaces<sup>19,20</sup>. Only the general features of the phenomenon are clear as yet; thorough investigation by those studying processes involving the interaction of vapours and solid surfaces is clearly required.

The Institute of Physical Chemistry, USSR Academy of Sciences

B. V. DERJAGUIN N. V. CHURAEV

The end of polywater. Extracts from the paper by Deryagin (Derjaguin) and Churaev, published in *Nature* on August 17, 1973.

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Recognizing that it is easier to reconstruct history than to learn from it, Franks, nonetheless, attempts the exercise. One lesson is that since competition is the driving wedge behind science, the race for

priority created the polywater episode; other bungles like it are inevitable, given enough money and freedom. But competition may not be essential. Other ways of getting results may be just as good, if not better. We know that there is an opposing tendency for investigators to collaborate, rather than beat each other to the punch. Competition, like polywater, may be merely an artefact of our times.

Robert Ubell is the American Publisher of Nature. During the "polywater years" he was Editor-in-Chief at Plenum, where both Felix Franks and B.V. Deryagin were his authors.

# Enchaining the power of catalysis

Herman Mark

The Chain Straighteners. Fruitful Innovation: The Discovery of Linear and Stereoregular Synthetic Polymers. By Frank M. McMillan. Pp.207. ISBN 0-333-25929-7. (Macmillan Press, London: 1981.) £17.

THE steady and slowly moving mainstream of science and technology is occasionally interrupted and enlivened by unexpected events which accelerate it and eventually change its direction into new and hitherto inaccessible domains. Popularly known as "breakthroughs", such drastic evolutions have, since the last war, occurred in mathematics through the advent of electronic computers, in physics as a result of nucleonics and laser optics and, probably best known, in the life sciences through the discovery of helices in the structure of proteins and of double helices in that of nucleic acids. This book relates in a captivating and intriguing manner the story of another breakthrough which occurred in the mid-1950s in organic chemistry and the closely related field of polymer science and technology

It started in the late 1940s when Dr Karl Ziegler, then Professor of Organic Chemistry at the University of Halle on the Saale, became Director of the Max Planck Institute for Coal Research in West Germany. Ziegler, already a well known and distinguished scientist at that time, had worked for years on a family of substances at the border of inorganic and organic chemistry known as metallorganic compounds and had specifically concentrated his efforts on the alkyl derivatives of aluminium. One particularly representative member of this family, triethylaluminium, is a colourless, volatile liquid, highly flammable and even explosive, requiring extreme skill in its preparation and handling. But these problems, a consequence of its high reactivity, attracted Ziegler rather than deterred him; and his experimental virtuosity allowed him to dominate this field for a number of years. In the early 1950s he observed that ethylene could be added to triethylaluminium in such a manner that hydrocarbon chains from 10 to 20 carbon atoms were obtained. Ethylene was, at that time, a readily available and inexpensive raw material of the petroleum industry and the resulting aliphatic chain compounds were valuable starting materials for detergents, emulsifiers and plasticizers. No wonder that several chemical companies — for instance, the Montecatini Company in Italy — started to negotiate with Ziegler about a joint evaluation of his invention. Evidently his new process was interesting and useful.

But the real sensation came a year later. As a result of a strange sequence of trial and error steps, Ziegler and his associates found that through the addition to the aluminiumalkyl of heavy metal salts ethylene could be rapidly transformed into linear, high molecular weight polyethylene. Even though there already existed a well established commercial process the ICI process — for the production of a non-linear and somewhat softer type of polyethylene, and even though a linear polyethylene - Marlex of the Philips Petroleum Company — was on the verge of becoming commercial, the Ziegler discovery acted like a bombshell. The complete novelty of the catalytic system, the mild conditions of the polymerization and the resulting simplicity of the equipment produced a rarely experienced rush of licences for the new process. Careful laboratory work with difficult materials, intuitive appraisal of unexpected results and energetic pursuit of all available avenues had created a new tool for the polymer chemist — the Ziegler catalysts and started a new and important branch of polymer technology.

But, to the delight and surprise of the reader, this is only part of the story of the book. Dr Giulio Natta, directing the Chemistry Department of the Polytechnic Institute of Milan, happened to be a consultant for the Montecatini Company and, in due course, became familiar with the existence of Ziegler catalysts. He applied

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The Nobel Prize winners — Ziegler (left) and Natta listen as Professor Fredga makes the presentation speech, Stockholm, 1963.

them on propylene instead of on ethylene and, of course, obtained polypropylene. Considering that, at that time, no high polymers of propylene were known, this was an important contribution to polymer technology. It was dwarfed, however, by Natta's discovery that the use of Ziegler catalysts

permitted the preparation of several species of polypropylene and other vinylpolymers differing from each other only by the steric arrangement of the substituents. Until that time stereospecific catalytic power had only been observed with natural enzymes: it is understandable, therefore, that the discovery of the existence of dozens of stereo-regulated polymers and their precise identification added to this commercial

success a major scientific sensation.

Dr Frank McMillan, as a contemporary of all these events, was manager of a large industrial research laboratory and has, therefore, acquired a special feeling for the intricate relationship between fundamental research and practical application.

Needless to say, the alluring commercial features of the new catalysts and their novel applications attracted, with increasing intensity, the interest of many large companies. As a result of their permanent and strong participation, there came the day when the predominant question was formulated: "What belongs to whom?" a question which has been debated for the last 25 years. Understandably, the litigations have led to several confrontations, and in this domain the author is a true master of ceremonies, distributing fame and blame with restraint and distinction. All this makes excellent reading: entertaining, instructive and sometimes even philosophical. In several instances he offers specific warnings to avoid certain mistakes in research and development and even in the recording of results. The clarity of his exposition should certainly convince readers to avoid such mistakes in future. But, very probably, in doing so, they will make others.

Herman Mark is Dean Emeritus of the Polytechnic Institute of New York. At the time of the discoveries described in the book, he was in contact with two institutes in Germany and Italy and contributed some ideas to the mechanism of Ziegler-Natta polymerization.

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# Jean Baptiste Rousseau revisited

Gunther S. Stent

The Double-Edged Helix: Science in the Real World. By Liebe F. Cavalieri. Pp. 196. ISBN 0-231-05306-1. (Columbia University Press: 1981.) \$14.95, £10.80.

THE latter-day metamorphosis of molecular biology from the esoteric speciality of a small band of aficionados into an academic juggernaut and billion-dollar industry, and its technical and moral implications, have not been slow to draw the attention of sociologists and ethicists of contemporary science. Indeed, the writing of books about the banishment of molecular biology from the Garden of Eden has become a minor cottage industry. The Double-Edged Helix by Liebe F. Cavalieri, a biochemist working at the New York Sloan-Kettering Institute for Cancer Research, is another contribution to this literature. Subtitled Science in the Real World, Cavalieri's book addresses the politics of the recent controversy surrounding the development of recombinant DNA technology. One of his chapters is entitled "Rousseau Revisited", and since Cavalieri makes no allusion to Jean Jacques, the philosopher, he appears to be revisiting Jean Baptiste, the poet, who was prosecuted and exiled from France in 1712 for libelling his colleagues.

As presented by Cavalieri, the situation is as follows. There are some people who believe that genetic engineering by recombinant DNA methods is dangerous and should be closely controlled. They are "thoughtful", "unusually frank" and "valiant"; they "have a conscience", "question neatly" and "testify" before government bodies "in the public interest". Other people, by contrast, believe that there is little or no danger in this enterprise and oppose strict controls on recombinant DNA research. They are "simplistic", "self-serving" and make "crusades"; they have "myopic vision" and form part of the "power structure", "smack of scientific elitism" and "lobby" before government bodies, "snowing" them with "massive campaigns" to satisfy spurious" and "inane" needs.

Moreover, many of these opponents of strict controls happen to be holders of the Nobel Prize, "an exceedingly dangerous device" that gives each of them "virtual limitless power . . . within his institution and among his colleagues": others are merely members of the National Academy of Sciences, that "tends to favor special interests", "does not represent the bulk of the national science effort" and has a president, or "high priest", who "muddies the water" and "breaches . . . canons of scientific propriety". I think there is little chance that many readers of Nature will find merit in this book. But it may be useful all the same to dissect and review briefly Cavalieri's main propositions.

1. Molecular-genetic engineering is morally wrong because "the natural gene pool of the earth [is] an inalienable birthright". Moreover, we must not cross the "natural genetic barrier between species which protects the integrity of the species" as is generally done in recombinant DNA experiments. This is not a political argument, as Cavalieri thinks it is, but a theological, non-utilitarian one that has meaning only within the Judeo-Christian tradition. Since God has obviously permitted the natural gene pool of the Earth to change over evolutionary time and allowed man to change it since Neolithic times, this proposition, if true, would present us with another paradox of theodicy. In any case, what it is we are and are not allowed to do genetically hinges on the hermeneutics of Genesis 1:26 versus Genesis 2:7 and the sense in which God gave man dominion over the animals. Hence the discussion must focus on whether or not our divine grant of "dominion" includes permission to alter the natural gene pool and cross species barriers. Genesis 9:1 is relevant here, in that the passengers of Noah's Ark provide the exegetically pertinent explication of "species". And as regards species crossing, Cavalieri's proposition is supported by Leviticus 19:19: "Thou shalt not let thy cattle gender with diverse kind; thou shalt not sow thy field with mingled seed". I believe it is possible to produce a rational argument according to which a devout Jew, Christian or Muslim should not undertake recombinant DNA experiments. In a secular context, however, the gene pool and species-crossing proposition is irrational.

2. Molecular-genetic engineering is potentially very dangerous, and hence should not be carried out, or, at least, should be closely controlled. This utilitarian argument is the centrepiece of the *The Double-Edged Helix* and, although it can be simply stated, it is actually quite complex. First, as far as the dangers themselves are concerned, they can be subdivided into short term — "immediate biohazards that could result from a laboratory accident" — and the long term — "irreversibility of the organisms themselves and the irreversible socioeconomic entrenchment that will result from the

successful use of recombinant organisms, regardless of their side effects". As for the immediate biohazards, there seems to be no disagreement regarding the possibility that such hazards may exist. What is under dispute is who, if anyone, is competent to assess these hazards and decide whether there is or is not a reasonable chance of averting them.

According to Cavalieri, the molecular biologists who are actually engaged in genetic engineering cannot be trusted to make this assessment, because their selfinterest causes them - Nobel Laureates, Academy members and just plain bench workers - to make dishonest risk appraisals and use their "clout" to sink even the timid guidelines by means of which prudent government administrators and legislative bodies sought to protect the common weal. So it is left to investigative reporters, consumer and environmental protection organizations, and "socialresponsibility-in-science" groups, whom Cavalieri cites mainly in support of his arguments, to identify the substantial biohazards associated with recombinant DNA. Of just what these hazards actually consist is, however, not - probably for lack of expertise - clearly or credibly spelled out. If Cavalieri's low opinion of the moral fibre of what he calls the "science community" were an accurate perception, the real world, being deprived of reliable expert opinion on vital scientific matters, would really be in serious trouble.

Fortunately I can recognize the scenario that has the legion of molecular biologists currently engaged in research using recombinant DNA techniques, carelessly risking the survival of mankind to satisfy their idle curiosity or venal cupidity, as merely a paranoid fantasy.

As for the long-term hazards, Cavalieri finds that in them lie "the most serious dangers of recombinant DNA technology". But, of just what these serious dangers consist Cavalieri spells out even less clearly than he does for the shortterm biohazards. He merely points to the history of twentieth-century technology, which shows that many developments originally thought to be benign later turned out to have unanticipated malign sideeffects. Thus Cavalieri calls on the journalist John Lear to remind us that Henry Ford's invention of the massproduced automobile, though it provided mobility to Everyman, turned out to deprive him of a livable habitat. And Cavalieri wants us to "remember the nuclear spills, and how we were reassured about the safety of nuclear power plants". So how does "the irreversible socioeconomic entrenchment of recombinant organisms" present a long-term danger? Because it will

provide technological fixes for past failures that cannot be rooted out at the source. Thus we try to find a technique for curing lung cancer while we continue to . . . advertise cigarettes, and we develop oil-eating bacteria to clean up oil spills,

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instead of redesigning oil tankers or reexamining our energy-intensive and wasteful economy or making a serious effort to shift to renewable and ubiquitous energy sources.

That in the socialist countries, where there is no advertising, cigarette consumption is even higher than in the capitalist world, and that work is already in progress to produce genetically engineered microbes intended to provide a renewable energy source which would do away with oil tankers altogether, is apparently not known to Cavalieri. But for him the most ominous long-term hazard is the application of recombinant DNA techniques to the human genome. He admits that there is the possibility of performing "gene therapy" on a number of hereditary defects. But Cavalieri finds that it is not "a high priority line of research to be chosen in preference to other directions" and its benefits pale in camparison with the spectre of eugenics, which 'received considerable support from industrialists like the Harrimans, Kelloggs and Carnegies". Though "its demise was aided by the repugnancy of emerging Nazism . . . with the development of new genetic techniques the eugenics movement in America could rise again".

To this it can be said, first, that whereas it is true that the project of improving the human "gene pool" by eugenic methods has fascinated many prominent geneticists, it is also a fact that despite the long-time availability of techniques (such as artificial insemination) for implementing eugenic goals by methods more humane than those practised in Nazi Germany, wide-scale eugenics has not come into use in any democratic society. So, there seems to be a firm (probably religiously rooted) resistance to eugenics, leaving its advocacy mainly to scientistic cranks. Second, and more importantly, it may be the case that any answer to "the classical question: who decides what is a defect?" could lead to procedures "clearly open to abuse". But, all the same, opposing on those grounds the use of diagnostic, prophylactic and corrective procedures in medical genetics reveals a lack of genuine empathy with and concern for people in the real world, since there are hereditary disorders that every person would judge to be defects with which no human being ought to be born. Thus Cavalieri's argument against genetic engineering from long-term hazards consists merely of general cant about Henry Ford, nuclear spills and the Nazis, and puts forward no specific prognosis that can be critically examined and discussed. Rather, just as does the argument from immediate biohazards, this argument, too, merely indicates a radical lack of faith in the honesty and wisdom of the leaders responsible for the management of our democratic society and of our scientific colleagues.

3. The potential benefits of moleculargenetic engineering are too small to offset the enormous risks. "The now familiar list of potential benefits that may accrue from

recombinant DNA includes . . . the production of insulin . . . antibiotics . . . vitamins and hormones . . . and . . . food crops. . . . Do we need them?". Cavalieri answers "no". As for insulin, a "thoughtful approach to the problem of diabetes ... was given by Harvard's Professor Ruth Hubbard" who declared insulin to be a "technological gimmick". She counsels that we should rather try to find "the causes of diabetes, which are, as with all other diseases, heavily influenced by social and environmental factors". And as for antibiotics, vitamins and hormones, in the United States we have already 20,000 pharmaceutical products in medical use, when "the World Health Organization has indicated that only 210 drugs would be sufficient to fill world health needs". And as for food crops, "we must not let our understandable sympathy for the hungry people of the world lead us into mistaking the cause of the problem, which is not one of production or quality but of distribution and utilization. The world now produces enough grain to feed everyone adequately". That is to say, abundant food is available to feed the hungry, if only the nations with undernourished populations would organize better politically and economically so that they can buy food from the affluent countries that waste their food surpluses anyhow. So "no real need has yet been brought forward to justify the serious ecological hazards of introducing major disturbances into the complex balance of things" by recombinant DNA methodology.

It is not necessary here to enter into a dis-

cussion of the merits of Cavalieri's claims. For even if these claims were just, the finitude of his list of potential benefits and his additional pronouncement that "we are no longer in an area when practical applications of scientific research are unforeseeable and the human consequences unknown" show a demagogic refusal to allow that what is sauce for the goose is also sauce for the gander. If it is the case that, as Cavalieri claims elsewhere, history teaches us that the long-term hazards of scientific and technological developments are always unforeseeable, he cannot in good faith allege that the time has come when all their benefits are foreseeable. Moreover, Cavalieri's competence to discuss, not ethics, but modern DNA research is put into question by his failure to mention the amazing advances that recombinant DNA techniques have brought to our understanding of the molecular organization of genetic structures in the past three or four years. An author of a book on recombinant DNA that appeared in 1981 who does not mention the discoveries of the fragmentation of eukaryotic genes and the mechanism of generating the diversity of antibody specificity, neither of which could have been made without the use of recombinant DNA methods, commands just about as little credence among biologists as one who, in the 1960s, would have failed to mention the discovery of the genetic code.

Gunther S. Stent is Chairman of the Department of Molecular Biology and Director of the Virus Laboratory at the University of California, Berkeley.

# A haunted house of cards

D.R. Newth

A New Science of Life: The Hypothesis of Formative Causation. By Rupert Sheldrake. Pp.229. ISBN 0-85634-115-0. (Blond & Briggs: 1981.) £12.50. To be published in the US in January 1982 by Tarcher, Los Angeles.

THE title of this book is misleadingly modest. The author is not content to propose only a new science of life, for he reassesses many features of the real world that have been revealed by natural science, and proposes that there exists a great conservative principle making itself felt as much, or more, by sub-atomic particles as in developing embryos or in the behaviour of human beings. The principle is that what happens, or has happened, can exert an influence that is without decrement in space or time upon future events of a similar kind. This influence acts to promote a repetition of what has gone before. The degree of similarity qualifying a living organism to respond to these persuasive messages appears to be conspecificity. Not all decisions or events, however, are susceptible to the principle of "formative causation".

The immediate recipient of the messages is a "morphogenetic field" which guides formal change in its associated "morphogenetic germ" until its prescriptions have been met and the "morphic unit" is finally co-extensive with the field. The morphogenetic field blends the experience of all previous similar morphic units by a process of "morphic resonance". Neither morphic resonance nor the obedience of the morphogenetic germ to the dictates of its morphogenetic field involve exchanges of matter or energy.

This, I understand it, is the burden of Dr Sheldrake's argument.

It is, of course, brave to expound in little more than 200 pages so revolutionary a denial of everything that empirical science has made seem probable. Nor should we deny some leniency to the holders of really way-out ideas. They lack the support of an established terminology, and the com-

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forting background of assumptions held in common with their readers. But they should at least try to be clear, at whatever cost to their credibility.

Dr Sheldrake writes as Mrs Bloom daydreamed, with no one theme rigorously explored before it sets off another which before resolution gives way to something else again. It would be unkind to suggest that this is a device for escaping from difficulties, but even readers who are wholly unsympathetic might welcome a clearer view of the author's position. For example, in discussing the limitations of morphic resonance he assumes that while past events can be effective now, future events cannot. While conceding that it is logically conceivable that they might be, he excludes the future on the ground of simplicity and remarks severely that "only if there were persuasive empirical evidence for a physical influence from future morphic events would it become necessary to take this possibility seriously". Apart from the ambiguity of introducing physical influences into a discussion of extraphysical phenomena, one is left wondering why the same severity is not applied to the past.

Indeed, Dr Sheldrake does believe that his ideas are capable of receiving support from experiment, but his proposals for experiments are curiously tentative and unsatisfactory. Thus

... if thousands of rats were trained to perform a new task in a laboratory in London, similar rats should learn to carry out the same task more quickly in laboratories everywhere else. If the speed of learning of rats in another laboratory, say in New York, were to be measured before and after the rats in London were trained, the rats tested on the second occasion should learn more quickly than those tested on the first.

Well, yes, but so they should without the London intervention, and any quantitative predictions in the operation of this hypothetical principle are so wholly arbitrary that the design of such experiments would be difficult indeed. Dr Sheldrake concedes this in his rather casual



Rupert Sheldrake — contribution to a happy state of confusion?

suggestions for a handful of investigations in each of which he describes a possible result *supporting* formative causation, but the opposite result is inconclusive. It would be a help if he could offer us predictions the failure of which would end the matter.

Anyone tempted to take formative causation or morphic resonance seriously should ask themselves why. A world haunted by messages from the past, some, like those from morphic units of extinct species, destined to vibrate eternally and in vain while seeking a morphic germ with which to resonate, may have a poetic appeal. Unfortunately it may also appeal to a perverse fear of scientific understanding. Dr Sheldrake explains early in the book

that while some outstanding biological problems are difficult, others are, in principle, insoluble — for example, those associated with evolution and with the origin of life. Neither, as it happens, is suitable for the operation of formative causation, since they are creative and unique rather than repetitive. But by the end of his exposition one reader had the distinct impression that intrinsic insolubility had its own attractions for him and that the hypothesis of formative causation was his contribution to a happy state of confusion.

D.R. Newth is Regius Professor of Zoology at the University of Glasgow.

# Selling newspapers and selling science

Eric Ashby

Reflections on Science and the Media. By June Goodfield. Pp.128. ISBN 0-87168-252-4. (American Association for the Advancement of Science: 1981.) \$9.

"JULES de Goncourt once wrote that . the newspaper bore the same relationship to a book as a whore did to a decent woman". A verdict, as June Goodfield says, "too harsh by far"; but as a caricature of the way some of the mass media deal with science it has an uncomfortable relevance. A news item is a one-night affair unless it attracts enough readers or the TV ratings to justify a follow up. As soon as the issue no longer stimulates the reader or the viewer it is dropped. Never mind that the issue is important, still unresolved and ought to be kept before the public: out it goes to make room for something more newsworthy. Of course there are honourable exceptions to this generalization, but as a rule news keeps no better than fish: indeed less well than fish, for fish can be put in cold store and still eaten with relish. News that has been put in store is worthless.

This is one of the reasons for the mutual suspicion between scientists and journalists. They work on different timescales. The scientist who publishes halfbaked findings loses the respect of his peers. The journalist who fails to publish his findings promptly, however half-baked they are, loses his job. And on the journey from the laboratory to the news-stand the information may get horribly distorted. The interview between journalist and scientist may have lasted an hour; the journalist has to chip away the reservations and complexities so that he can fit the story into half a column; the sub-editor (the worst culprit of all) slices chunks out of the journalist's copy and adds a sensational headline which must often sicken the journalist as well as infuriate the scientist who in good faith has explained his work.

And the outcome: a wider alienation between science and the media.

This didn't matter in the days before the public became the patrons of science. It would not matter today if everyone read the New Scientist or the science correspondents in the quality newspapers, or even watched science programmes on the BBC. But they don't, and they pay taxes some of which support science. What should they be told about science?

In the United States the presentation of science on TV is much less satisfactory than it is in Britain, and science in the massmedia newspapers is no better reported than it is in Britain. The only science that is "sold" to the public is likely to be scandalous (for example the thalidomide affair) or open to ominous speculations (genetic engineering) or fashionable (pollution, in the 1970s). What is urgently needed is a much better public understanding of what science is about, how it is done and what consequences it has for society. These are difficult matters to put across and there's no money to reward those who try to do it.

It was, therefore, an excellent idea for the American Association for the Advancement of Science to commission an essay on science and the media, and a brilliant choice to offer the commission to June Goodfield, who already has an international reputation for her interpretation of science to the so-called lay reader. With first hand experience in the two professions of journalism and science and a sympathetic understanding of the constraints under which both these professions work, she has offered a clear and in places refreshingly provocative analysis of the "uneasy relationship" (as she calls it) between the two professions.

She rejects the view (still held, alas! by some scientists) that the great bingoplaying, football pools, top-of-the-pops majority don't need to be told about

science and wouldn't understand it anyway. They do understand the science stories that do get into the mass media: if this were not so, the science stories would not appear there at all. But this does not mean that science reporting must be sensational. Members of Mechanics' Institutes in the nineteenth century responded with enthusiasm to the journalistic essays of Faraday. Readers of the tabloid press were enthusiastic about science reporting by J.B.S. Haldane in the 1920s. So, there is a strong case for science journalism at this very popular level to be accurate, enlightening and responsible. What constraints stand in the way?

For the mass circulation newspapers the constraints are that the news item must arouse immediate interest, it must be grasped at first reading, and it must have an element of novelty. (How close to the bone was the analogy of Jules de Goncourt!) If papers don't sell they go out of business. Released from these three constraints they would not sell.

For television news, as contrasted with sustained scientific programmes such as The Ascent of Man and Life on Earth, there are similar constraints. A Select Committee report (on hazardous wastes for instance) is published on a Tuesday. It is, let us say, 150 pages long. On Tuesday evening it will have to be reported "on the box", with a verdict on its merits or (preferably) its deficiencies, mustered that afternoon over the telephone. In Britain some of the sustained programmes are splendid. This is possible (as June Goodfield explains) because the producer is "totally responsible for the content and impact of the work". He doesn't have someone else in the hierarchy breathing down his neck all the time. In America it is otherwise; there "the long arm of sponsorship" reaches down even to the details of the production. Even public television, where there have been some successes, seems constrained to plug the wonders of science rather than the experience of doing science and the effects science is having on the way people live.

The scientist, too, works under constraints that block his contacts with the public. His peers, higher in the pecking order of distinction, are breathing down his neck: too much (or, just as bad, too successful) popularization, and the whisper "not altogether sound, you know" echoes in the academic groves. News is enlivened by human interest, so the media like a little autobiography to be brought into a science story (hence the success of James Watson's The Double Helix). But it is the aim of scientists at work to be self-effacing; they never publish their false starts, their failures, their own reactions to the research they do: all these are filtered out before the paper is sent to the learned journal to be published. The chief constraint is the necessity imposed by the conventions of scientific research not to reach premature conclusions, not to

advance to a position from which you may one day have to retreat, and never, never, in reporting research, to extrapolate beyond your data to broad speculations. If your research does have social consequences, discussion of these has to be put into different journals addressed to a different readership. This is one of the difficulties science reporters have to contend with.

June Goodfield illustrates the difficulties in the way of bringing journalists and scientists on to the same wavelength by four examples. There was the scramble of reporters to make a sensation out of the affair of Summerlin's painted mouse. In their haste to get a story out, all but one of them got the story wrong. Only one journalist, Gail McBride of the Journal of the American Medical Association, took the trouble to understand the whole story, and (of course) by the time she published, four months after the event, it was no longer news. Then there was the worldwide, and at times hysterical, publicity following the Asilomar conference on recombinant DNA. There was an even more hysterical exhibition over Rorvik's false claim about a cloned man, exposing the venality of a publisher who contrived to create a bestseller out of the phoney book. And there was the example of investigative iournalism at its best: the sustained campaign to expose the cover-up over thalidomide.

With great skill June Goodfield attempts a summing-up. Both scientist and journalist begin on common ground: it is their business to discover and to publish the truth. Neither profession has an explicit code of ethics, nor a controlling body which (like the General Medical Council) can deprive a practitioner of his right to practise. In science, peer opinion provides a crude, but on the whole effective, mode of control. Shoddy work is not condemned in bitchy articles, such as Leavis wrote about Snow; it simply sinks without trace.

In journalism the implicit code of ethics is weakened by a pervading hypocrisy. It is a scoop to publish a secret government document illegally obtained; it is also a scoop to expose someone outside the profession who has obtained a secret document for his own ends. A contractor who bribes a politician is fair game for publicity, but not a journalist who bribes a witness. June Goodfield wants journalists to tighten their code of ethics but she would like them also to assume some responsibility to act as critics of the ethical implications of science. The need for this arises because scientists do not have a cloud of critics hovering round them, as novelists and dramatists have, and they deliberately dissociate their work from its ethical overtones.

As for the scientists, she wants them to take the trouble to sympathize with the constraints under which journalists have to work, to be less arrogant and more co-

operative, to realize that journalists are an essential channel of communication between themselves and the public on whom they depend for a livelihood. She wants devices for disseminating information in suitable form to the media. She wants the interpretation and communication of science to be taken more seriously as disciplines in universities, so that a student could find training for science-writing.

Her main suggestion is that there should be critics in science who play roles like those played by critics in music, literature and the arts, critics "in the accepted, oldfashioned meaning of the term". This is the one point in June Goodfield's essay with which I disagree: for two reasons. First, the achievements of science are published normally as papers in journals, not as books. (For books there is a mild critical apparatus already, e.g. in the review columns of Nature and other science magazines.) Second, the scientific content of research papers is effectively criticized by the use made of the papers by other scientists. Research of high quality is quoted. Research of poor quality is disregarded. So I see no merit in having professional science critics. If all critics were like Walter Lippmann or Edmund Wilson or Donald Tovey, that would be fine. But critics now abound like carrion flies, swarming over the work of people engaged in the desperately difficult professions of literature and art, dismissing, with a patronizing drawl, such as one hears on some radio programmes. creative work they would be quite incapable of doing themselves. God preserve us from critics like that in science. Nor will they be needed while there are scientists about who have June Goodfield's brilliant capacity to work at the interface between science and the humanities

Lord Ashby is Chancellor of Queen's University, Belfast, and a Fellow of Clare College, Cambridge.

## The measure of man

P.B. Medawar

A History of the Study of Human Growth. By J.M. Tanner. Pp.499. ISBN 0-521-22488-8. (Cambridge University Press: 1981.) £30, \$69.

PROFESSOR Tanner has made important contributions to the practice and methodology of anthropometry; one thinks especially of his studies of secular changes in human growth rates and in landmarks such as age of onset of sexual maturity in the two sexes. He is well known also for his advocacy of the cohort method in the analysis of human growth (the "longitudinal" method as opposed to the more familiar "cross-sectional" method

which has the disadvantage that curves of growth tend always to be confused by curves of distribution).

It is not usual for practitioners of a science to write of its history, but if it comes off, as it does here, the result is a triumph. Tanner could hardly do otherwise than begin with the voluminous and in the main tiresome works by or attributed to Aristotle: instead of calling attention to his more egregious and least easily explicable blunders, such as the belief that human semen is infertile until the age of 21, Tanner makes the best of Aristotle by finding grounds for the neat and convincing inference that "in 350 BC children were maturing at approximately the same rate as now".

Among Tanner's most interesting passages are those that deal with the hebdomadal tradition: the number-magic that found expression in the belief that the body is divided into seven parts and the life of man into seven stages each seven years long.

The mystique attached to the seven-fold system makes reliance on the truth of any actual age impossible however; both at the time and for centuries later there is a tendency for all ages to be rounded to the nearest hebdomadal number.

Tanner is a learned and widely read man, and his book is full of fascinating historical and sociological insights:

... the growth of children of the manual labouring classes in England in the 1830s, and even the 1870s, was still more depressed than that of the poorer groups in some of the underdeveloped countries nowadays ... we should not be too surprised. Indeed children of slaves in the plantations of the southern states of America at this time ... were taller (by some 2 to 5 cm) than contemporary children of manual abourers in England (although nowadays the heights of children of European and of African descent living under similar economic circumstances are almost identical).

Tanner devotes some amusing paragraphs to passages from J.J. Virey ("that would have done credit to Baden Powell") professing to demonstrate the debilitating effects of masturbation. J.J. Virey (1775–1846) was a pharmacist who contributed to the Dictionnaire des Sciences Médicale.

Although the book is mainly about human growth, Tanner casts a wider net and manages to make mention of D'Arcy Thompson, who had little to say about human growth. As an admirer of D'Arcy's, I can vouch that Tanner's placing and appraisal of him are exactly right — "a late flower of the Renaissance" who wrote superlative prose.

This is an excellent book which may be read with advantage by all biologists interested in the history of ideas — and should certainly be read by sociologists and social historians as well.

Sir Peter Medawar is Head of the Transplantation Biology Section at the Medical Research Council's Clinical Research Centre, Harrow, Middlesex.

## Education in science, science in education

Nevill Mott

Science in Schools: Which Way Now? By Richard Ingle and Arthur Jennings. Pp.185. ISBN pbk 0-85473-100-8. (University of London Institute of Education/NFER-Nelson: 1981.) £5.30.

THE authors of this book give an account of what has been happening in science education in the UK and of what, in their opinion, ought to happen now. One of them graduated in physical sciences, the other in biology; both had considerable experience of school teaching before moving into the field of teacher training and educational research. The emphasis of their book is on "science for all" and it gives as clear a discussion as any I have seen of the objectives to be achieved by giving all children, up to the age of 16, considerable time for science. What the authors discuss rather little, in that they do not present it as a separate problem, are the needs of that minority who after further education are going to make use of their science, as engineers, technicians, research scientists and doctors.

The rise of general science in the interests of science for all, and its subsequent decline up to the time of the change to comprehensive education, is described here, as it is perhaps more fully in the chapter "The General Science Movement" in E.W. Jenkins's book From Armstrong to Nuffield (John Murray, 1979). In the years between the two wars, Ingle and Jennings say that the move towards general science was based on the notion that science should be for everyone, not just for the embryonic professional scientists (which presumably includes engineers, doctors and others), and that it should form an essential part of the general education of all young people. But in practice, they add, general science was found to be "a scrappy mixture of physics, chemistry and biology". Certainly, in the immediate pre- and postwar periods it enjoyed little prestige as a university subject, in comparison with the specialist honours degrees. Moreover in the post-war period, policy-makers, conscious of the role of science in the war and of the perceived need for qualified scientists and engineers (OSEs) in industry, had little use for general science. Two authorities, quoted by Jenkins (p.99) wrote that

An improvement in . . . school science teaching is a requirement for the continued existence of this country as a leading scientific and industrial nation. As a first step in this direction general science should be abandoned.

Ingle and Jennings report that in the 1950s, in independent and grammar schools, general science was declining fast and two or three specialist O-levels were the norm. However, in the secondary modern schools, they say, teacher training colleges serving them were sufficiently independent

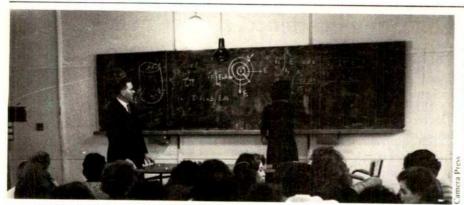
from universities to help teachers make the science more child-centred and less academic than in grammar schools. Unfortunately these teachers were rarely encouraged to publish their methods, "so that their experience and wisdom died with them".

A second chapter deals with "the curriculum development era", starting in 1960, and particularly the Nuffield Science Teaching Project, beginning in 1961-1962. This aimed to produce material in the three separate sciences, initially in the age group 11-16, for children in the selective grammar schools, and equally suitable for both future science specialists and others. In all three projects it was the aim that children would learn science largely through experimental enquiry, with the guidance and support of the teacher. The reception of Nuffield in the schools is described, as is its influence even in schools where the whole package was not accepted. Two criticisms of the original Nuffield scheme have been that the work was very demanding, even for the more able pupils, and that neither the O-level nor A-level schemes went far in illustrating the social relevance of science. Later projects for the less able child are described, including Nuffield Science 13-16 (now being published), the Association for Science Education's (ASE) project for "Less Academically Motivated Pupils" (LAMP), and also the much more demanding Schools Council's Integrated Science Project (SCISP), which with a time-table allocation equivalent to that for two O-levels brings together the three sciences with time for ample attention to be paid to their social aspects. Also described is the Schools Council's Project Technology, which had the aim of helping pupils to understand the importance of engineering in our way of life. In spite of the expenditure of £300,000, this project, they say, has had little impact; perhaps this is because science teachers teach best what they know best, and very few science teachers have been trained as engineers. In this context, the authors include an apposite quotation from B. Prescott:

Perhaps one of the greatest weaknesses of science teaching is that it is science teachers who do it — science teachers who have been initiated into the profession by science teachers and they teach courses written by scientists and science teachers.

But if this is a weakness, how is it to be corrected?

In chapters on aims and ideals in science education, and on hopes for the future, Ingle and Jennings identify themselves with the swing back towards general science and science for all, and away from the objective of producing QSEs, a movement which has undoubtedly taken place in the last decade. Reasons for this



Physics for sixth-formers — in the 1980s will they receive the grounding they need?

are perhaps the introduction of comprehensive schools, the unfavourable image that science and technology has sometimes presented and a widespread reaction against the so-called elitism of conventional scientific education. An extreme example of this reaction was the issue by the ASE of its discussion document "Alternatives for Science Education" (1979) which two years ago sent a shock wave of dissent through more conservative circles.

In this document it was claimed that science education as it is now is elitist, designed for the minority intending to make a career in science and against the interests of the majority. Alternatives were suggested, some of which seemed to propose teaching more about the role of science in the modern world than the practice of science itself. The ASE's most recent document, "Education through Science", issued in August 1981, puts forward a more moderate policy for the future which does not differ much from the proposals in the book under review, and which indeed carries widespread support. It is proposed that in all schools for children between 11 and 16, one-sixth of school time should be spent on science this would correspond to the time spent on two O-levels - and that integrated courses including physics, chemistry and biology, tit-bits of other sciences and coverage of social implications would be devised. This, it is claimed, would avoid irrevocable choices at 13, which so often cut children and particularly girls off from careers in science and its applications. Also it would avoid the lack of balance between science and the humanities which the study of three separate science subjects is said to involve.

Thus science education, as mapped out in this book, should be intended for all children. Rather than summarizing the aims set forth by the authors, the flavour of their argument may be given by the following quotation from a CSE project report which they include in the book:

My project worked very well and I'm pleased because (i) I got alcohol from paper which I thorught was never possible, (ii) because I used some new aquipment which I've never herd of let alone worked with. Another thing I was pleased about was there was lots of experiments and if there was anything I wanted to know there was

book's at my finger tips so there wern't any time lost. If I had a lot more time what I would like to make is a lot more alcohol and do lots of flame tests because I only made about Icm<sup>3</sup> of pure alcohol. So I could not do much, I would allso like to find how much yeast is necessary to ferment it propley yet let the alcohol burn propley. I would also like to know if it was the yeast that stopped it burning. I would also like to learn how to control the heat when distilling because that a mistake I made.

Curiosity, ability to think and to form ideas of what science and technology are about are to be emphasized. The authors recognize that "there are many young people who will leave school to seek employment in some field of science and technology, and that these will need a more extensive knowledge of science". Reference is made to "the more demanding courses that may be necessary to engage their minds and prepare them for sixth form studies". The italics are mine. Little discussion is devoted to the vexed question of setting or streaming for these more demanding classes. The authors give the impression that, although they realize that setting in science subjects (or avoidance of science by the less talented) is almost universal, at any rate from 14 to 16, they hope that mixed ability teaching will spread. But this book does at least recognize the needs of the talented.

Two years ago the Education Committee of the Royal Society issued a discussion paper entitled "Science and the Organization of Schools in England; Implications for the Needs of Talented Children" Here it was taken for granted that setting for science and mathematics was in the interests of talented children, that they needed to be with children of like ability and to make progress at their own rate. The fear was expressed that in small comprehensive schools, particularly if falling rolls should make them still smaller, there would not be enough children to form an O-level class. It also defended the retention of three O-levels together with a choice of subject, among other reasons because teachers can teach best what they know thoroughly. It is true that almost any reform is bad if it does not increase the teacher's confidence in his mastery of his subject; for this reason Ingle and Jennings rightly stress the need for in-service training, preferably school based.

If school education goes the way recommended by Ingle and Jennings, we can envisage a future as follows. All children will spend one-sixth of their time on science up to the age of 16. There will be a common examination system, unifying CSE and O-level, but separate questions or even papers of appropriate difficulty may be set. The separate teaching of physics and the other subjects may disappear, except perhaps in the independent sector. There will be a common core; the lower ability groups will not have vague talk about science fobbed off on them but will be exposed to some real science. Ideas on the teaching of the less talented will develop, and some success is expected. Setting, however, is likely to remain. The experience with SCISP shows that talented children who have done the equivalent of two O-levels do not have difficulty with A-levels as they now are. Whether more science teachers able and willing to teach all three sciences will be found, only the future will show.

We have here a way forward which, I believe, may well be in the interests of the average child and also of the more talented child who does not want to specialize in science. But for future scientists, I have doubts. Probably a wider education, cutting down in the time spent on science up to the age of 16, will not do them any harm. On the other hand, if they are held back, and not allowed to progress as fast as they can, they may become disillusioned and even give up the subject. How much do we want to keep the most talented back, in the interests of the majority, or of general education or for any other reason?

If the authors of this book do not see this as a major problem, they have much of interest to say on many other matters. One is the need for collaboration between the teachers of science and of mathematics; another, perhaps more original, is the help that historians ought to give in teaching the history of science. Another is the value of education in science towards learning to express oneself clearly. Finally, the authors quote Richard Peters as writing (in 1972) that "our state of ignorance with regard to teaching is comparable to that of the Greeks with regard to medicine or meteorology". Ingle and Jennings end their book by expressing the hope that systematic school-based research will hasten the day when science teaching, while remaining an art, will become more of a science. And as a consequence, they hope, as we all must, "that the promotion of teachers might then begin to depend less on confidential references and more on soundly based competencies".

Sir Nevill Mott was Cavendish Professor of Physics until his retirement. He was a member of the Crowther Committee on education from 16 to 18, and has chaired various advisory committees for the Ministry of Education and for the Nuffield School Science Project. He is now a member of the Royal Society's Education Committee.

# **Gyrotropic Waveguides**

Paul Hlawiczka

December 1981/January 1982, x + 96pp. 0.12.349940.2 £12.80 (UK only)/\$26.50

The book includes discussions of the gyrotropic medium followed by various configurations of the gyrotropic waveguides and ends with a brief survey of the application possibilities at optical wavelengths at the time of writing. *Gytropic Waveguides* should therefore prove stimulating to research workers in optical communications and integrated optics, although it should also appeal to other scientists inerested in guided waves

# Multivariable and **Optimal Systems**

D.H. Owens

October/November 1981, x + 300pp. Cloth: £22.80 (UK only)/\$47.00 0. Paperback: £8.00 (UK only)/\$16.50 0.12.531720.4 0.12.531722.0

Most of the material published here is presented at a high level with emphasis on generality and mathematical rigour, yet the underlying concepts are essentially simple and the most applicable parts of the theory can be understood without using sophisticated mathematics. This text aims to provide a comprehensive, up-to-date and consistent treatment of the minimal foundations of those parts of the field with greatest relevance to practical design work. It enables the reader to tackle working design examples and lays the conceptual foundations for further study. All this is done using only undergraduate engineering mathematics.

Reprinted from Advances in Physical Organic Chemistry

## Stability and Reactivity of Crown-Ether Complexes

F. De Jong and D.N. Reinhoudt

October/November 1981, vii + 164pp. £12.80 (UK only)/\$31.00, 0.12.208780.1

This is the first of a new sequence of occasional reprint chapters extracted from the Advances in Physical Organic Chemistry series. Predominantly paperbound and priced inexpensively for the individual purchaser, they will give the specialist rapid access to selected articles of particular interest.

This book presents a critical evaluation of the physical organic chemistry of crown-ether complexes with organic and inorganic guest molecules. By analysing the parameters that influence the thermodynamic and kinetic stabilities, the reactivity, chiral recognition by chiral crown-ethers, and enzyme-type of catalysis, the authors draw some general conclusions about this subject.

# The Downy Mildews

edited by D.M. Spencer

October/November 1981, xxii + 636pp., £49.60 (UK only)/\$119.50 1.12.656860.X

The aim of the book is to present an integrated and authoritative statement of our present knowledge of the downy mildews. It is in two parts and the first chapters provide general reviews of the history, taxonomy, distribution, epedemiology, host range, genetics, breeding for resistance, host-parasite relations and control of the downy mildews. The second part then deals with the downy mildews of individual crops or commodity groups.

## **Blackflies**

The future for biological methods in integrated control

edited by Marshall Laird

October/November 1981, xii + 346pp., £28.20 (UK only)/\$58.00 0.12.434060.\$ £28.20 (UK only)/\$58.00

Leading specialists in all fields connected with the description of and solution to the blackfly problem have contributed to this book; classification, zoogeography, ecology and physiology are all examined. It is a comprehensive reference work on the background and control of blackflies, and it also provides a thorough description and appraisal of the most up-to-date discoveries and ideas on biocontrol.

# Advances in Parasitology

edited by W.H.R. Lumsden (Senior Editor), R. Muller and J.R. Baker

October/November 1981, x + 226pp., £16.40 (UK only)/\$39.50 0.12.031719.2

This long-running series has gained a reputation for its detailed and original reviews on a variety of subjects within the field of parasitology, Volume 19, the latest in the series, deals with three groups of parasites — which are not commonly dealt with in review articles. The present editors have broadened their policy to admit for review any group of organisms whose members follow the parasitic mode of life, emphasizing areas of parasitology which have advanced significantly at the time of writing.

## Genetic Consequences of Man-Made Change

edited by J.A. Bishop and L.M. Cook

September 1981, xiv + 410pp., £23.60 (UK only)/\$57.00, 1.12.101620.X

Contents

Contributors. Foreword. Preface. J.A. Bishop and L.M. Cook: Genes, phenotype and environment. J.A. Bishop: A NeoDarwinian approach to resistance: examples from mammals. R.J. Wood: Insecticide resistance: genes and mechanisms. R.J. Wood and J.A. Bishop: Insecticide resistance: populations and evolution. D.R. Lees: Industrial melanism: genetic adaptation of animals to air pollution. M.R. Machnair: Tolerance of higher plants to toxic materials. J.A. Barrett: The evolutionary consequences of monoculture. J.R. Saunders: Human impact on microbial evolution. R.J. White and R.M. White: Some numerical methods for the study of genetic changes. Index.

# Advances in Microbial Physiology

edited by A.H. Rose and J. Gareth Morris

September 1981, viii + 266pp., £22.40 (UK only)/\$54.00 0.12.027722.0

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Gerhart Drews and Jurgen Oelze: Organization and differentiation of membranes of phototropic bacteria. Howard Bussey: Physiology of killer factor in yeast. A. Fiechter, G.F. Fuhrmann and O. Kappeli: Regulation of glucose metabolism in growing yeast cells. P.D.J. Weitzman: Unity and diversity in some citric acid-cycle enzymes. Author index. Subject index.

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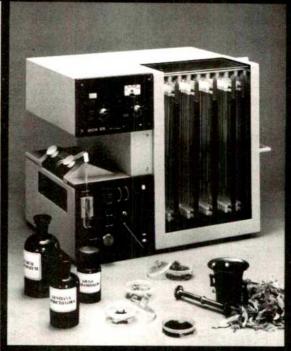
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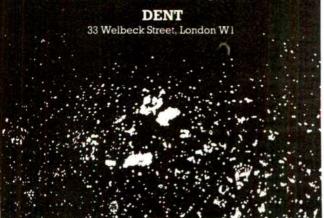
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# The eighteenth-century generation game

Christopher Lawrence

Matter, Life and Generation: Eighteenthcentury Embryology and the Haller-Wolff Debate. By Shirley A. Roe. Pp.214. ISBN 0-521-23540-5. (Cambridge University Press: 1981.) £16, \$32.50.

AT FIRST sight the eighteenth-century debate on the nature of generation and embryological development might appear to have been a parochial affair. The path from fertilization to birth, however, was by no means a simple scientific conundrum calling for a straightforward empirical enquiry. As Professor Roe displays so clearly in this excellent book, generation was a hinge on which turned alternative cosmologies. Different theories of development brought into conflict incommensurable universes. Professor Roe has therefore used this particular study as a case history to illustrate some more general philosophical points about the nature of scientific enquiry and explanation.

In the eighteenth century there were two possible views on generation which were scientifically respectable. Preformationists held that embryos pre-existed in either the semen or the egg and these embryos in turn, like a nest of dolls, contained intact the next generation in their germinal material, and so on. This theory, first fully articulated by Malebranche in 1674, had considerable advantages over its rivals. The scientific revolution had virtually swept the intellectual field by the late seventeenth century. It left behind only matter, motion and — after Newton — force as the fund-amental explanatory principles in the cosmos. Preformationism therefore explained the puzzle of why it was that the embryo developed in the way it did, rather than crediting the possibility that matter in motion could somehow give rise to organized material.

This latter eventuality was embraced by the epigenesists, who held that the embryo developed form and parts from where there

Chicken development — detail from Fabricius's De Formatione Ovi et Pulli (Opera Physica Anatomica, 1625).

had been neither form nor parts before. The most famous epigenesists of the eighteenth century were Maupertuis, Buffon and Needham, and in the world of belles-lettres the philosopher Denis Diderot. All of these thinkers circumvented the problem of formal development by postulating that matter was innately active and not the passive servant of other forces. The preformation -epigenesis dichotomy therefore was not a simple scientific schism. Rather, on the one hand lay the divinely formed embryo, special creation, a meaningful universe, and thus a Christian cosmology and salvation. With epigenetic development lay chance, purposelessness, Lucretianism and extinction.

In the middle of the eighteenth century this debate was rekindled by the pious, Newtonian, Professor of Anatomy at Gottingen, Albrecht von Haller and the rationalist, upstart physician, Caspar Friedrich Wolff. Haller espoused preformationism and Wolff epigenesis, and for ten years they discussed the issue in print and in private correspondence. Two factors make the debate particularly interesting. First, they both conducted a great deal of detailed empirical research on the development of the hen's egg, attempting to discover or refute whether the chicken came first so to speak. The debate thus turned on complex technical questions such as the appearance of the heart, the gut membranes or the yolk sac vessels. Second, Wolff was no ranting atheist. Rather, he too was a deeply pious Christian, but one who had begun with his feet in quite different metaphysical starting blocks to those of Haller. For Wolff, the laws of motion observed by matter had been created by God in the first place. Thus, epigenetic development was, in a way, preformationism one stage back.

Professor Roe unfurls this dialogue, or rather these two monologues, and shows clearly that, given the metaphysical corners of the protagonists, neither was going to get near enough to strike a blow. Where Wolff saw a heart developing and new bits forming, Haller saw a previously transparent structure becoming denser, coloured and demarcated from its surroundings. Professor Roe reveals these aspects of the debate with faultless precision founded on superb scholarship.

What is disapppointing, however, is that she stops short at either end of the argument. At the observational end she offers no discussion as to whether Haller and Wolff were interpreting differently the same data or whether they were actually seeing the world differently. Professor Roe never says whether Haller ever disagreed with Wolff's embryological drawings and denied that they represented reality, or whether Haller only disputed the meaning

of agreed observations. Neither is it clear whether Haller produced any drawings of what he saw. At the metaphysical end of the argument, Professor Roe seems to suggest her task is over when she has related a scientific debate to more general metaphysical principles. Having shown that the world rests on the back of an elephant, she neither asks if the elephant itself rests on anything or, if not, shows why her explanation of the Haller-Wolff debate might be sufficient once the metaphysics have been invoked. The epigenesispreformationism debate is therefore still a somewhat circumscribed area in eighteenth-century science. After this book, however, historians will need to perambulate a much extended perimeter fence.

Christopher Lawrence is Historian to the Wellcome Museum of the History of Medicine, London.

#### Battle over bacteria

G.D. Heathcote

The Fischer-Smith Controversy: Are There Bacteria! Diseases of Plants? Phytopathological Classic No.13. Translated and prepared by C. Lee Campbell. Pp.65. ISBN 0-89054-014-4. (American Phytopathological Society, 3340 Pilot Knob Rd, St Paul, Minnesota: 1981.) \$8.50.

PROBABLY few plant pathologists will make the time to read this pamphlet, which consists of little more than seven review papers published in Germany between 1897 and 1901, but the American Phytopathological Society did well to publish it. Perhaps they did so because it has a plot which could not fail to appeal to the American spirit. It tells how Erwin Smith, originally a poor farm boy from Michigan, battled (with words only of course) against the academic might of the classically trained Alfred Fischer, once an unsalaried lecturer in botany at the University of Leipzig, at the time when plant pathology was almost a German science.

The debate as to whether or not bacteria can be the direct cause of disease in plants stimulated the two protagonists into making bitter and personal attacks against each other. Smith's statement regarding part of one of Fischer's lectures: "It is seldom in a genuinely scientific book that one finds so many unwarranted assumptions and serious misstatements in the space of a single page ..." would undoubtedly have infuriated Fischer, and attacking Smith he wrote: "... after experiments of that kind ... no one will think badly of me that I had not sought further statements in the American

literature when I wrote my lecture".

Fischer was convinced that bacteria are only saprophytic upon tissues already broken down and that they cannot penetrate undamaged cells. Smith quoted experiments showing how bacteria can dissolve cell walls and that they can enter undamaged tissues, for example pear blight bacteria can penetrate through nectaries. In the end nobody won as such. Smith died in 1927 after a long and distinguished career, and although Fischer eventually suffered from depression and committed suicide in 1913 his professional stature was not diminished by the controversy.



Erwin Smith, farm boy turned plant pathologist. In 1899 he wrote of Fischer "...he garbles and misrepresents, charging other men with being stupid blunderers ...".

Nonetheless, the debate proved to be a significant event in plant pathology. Smith's view became accepted, and plant pathologists began to work seriously on bacterial diseases. Today between 180 and 200 species of plant pathogenic bacteria are recognized, causing serious economic loss throughout the world. They are unable to penetrate the plant cuticle and many require wounds to gain entry into the host plant but some enter through natural openings. Many are windblown or disseminated by splashing water, and at least 60 species can be carried by insects, for example fire blight of apples and related species is carried by honey bees.

The statements some of us make today are usually shorter but they are no more reasonable than those made by our predecessors. I can well remember the disbelief when it was first suggested that nematodes can carry virus diseases, and some of the diseases I thought to be caused by the viruses have been shown to be caused by mycoplasma-like organisms. Like so many controversies of the past, the Fischer-Smith debate repeats the lesson that in plant pathology, as in all of science, dogmatism is dangerous.

# Test tube to womb: ethics and politics

R.V. Short

From Chance to Purpose: An Appraisal of External Human Fertilization. By Clifford Grobstein. Pp.207. ISBN 0-201-04585-0. (Addison-Wesley: 1981.) \$17.50, £11.50.

In this book Dr Grobstein, Professor of Biological Science and Public Policy at the University of California, San Diego, seeks to describe for the general reader the technical procedures involved in human in vitro fertilization, and the social and political implications of this work. We are told that Dr Grobstein is internationally known for his research in developmental biology, and so we are entitled to expect a good read, and a penetrating analysis of present developments and future prospects.

The book turns out to be little more than an annotated commentary on the report of the US Ethics Advisory Board on Research Involving Human In Vitro Fertilization and Embryo Transfer, which was published in 1979. Fortunately this report is reprinted as an appendix, and for those not already familiar with it, it will form by far the most interesting part of Grobstein's book. Joseph Califano, the former Secretary of Health, Education and Welfare in the Carter administration, deserves considerable credit for setting up the Ethics Advisory Board, alas now disbanded, and charging such a distinguished group of scientists, lawyers, theologians, clinicians, ethicists and administrators with an in-depth investigation of the whole subject of in vitro fertilization. The way the Board went about this task was commendable. They commissioned manuscripts from leading experts known to have views on the matter, and held public hearings around the United States, with live radio and television coverage, to which anyone could give testimony, and at which the experts were cross-questioned by the Board. All the written evidence was then published, together with the incisive, common-sense judgements of the Board, who must have been greatly indebted to their chairman, a lawyer, James C. Gaither, for producing a consensus report from a Board that itself embraced such widely divergent views. The conclusions were neither remarkable nor controversial. The human embryo is entitled to profound respect, but this does not necessarily encompass the full legal and moral rights attributed to an adult individual. Therefore, a broad prohibition of research involving human in vitro fertilization is neither justified nor wise. Federal support for such work would be ethically acceptable, and there is a need for more research in order to assess the risks to mother and offspring.

The Board managed to steer a course between the Scylla of those such as the President of the Massachusetts Council of

Rabbis, who thundered that "Further in vitro experimentation could tend to eliminate the need for the human family and turn humanity into a zoo of fertilized and fertilizing animals", and the Charybdis of the brothers Seed (sic) who offered to buy fertilized eggs flushed from the uteri of women donors, and transfer them to the uteri of infertile recipients - at a price. Nevertheless, the publication of the Board's findings in the Federal Register on June 19th 1979 produced a storm of written protest from, one suspects, the Right to Life group. Secretary Califano took no action on the Report's recommendations, and shortly thereafter resigned. The Ethics Advisory Board was then disbanded because Congress established a new "President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research" that does not seem to have addressed itself to the problem, and America changed Presidents.

Since that time, nothing more has been heard of the Board's report. It seems to have sunk without trace, presumably too hot for a right-wing administration to handle. But thereby went an honest attempt to reach a consensus view by public debate of a contentious issue, and much time and money was wasted in the process. It is sobering for scientists to realize that even if an issue is scientifically, socially and ethically acceptable, it will receive no governmental support unless it is politically expedient. But Everyman will have the last say; new clinics are opening up around the world, and the number of successful births following in vitro fertilization is now into the teens. Even the President of the United States cannot halt such progress, although the paucity of governmental funding for the back-up research that is so urgently needed can significantly delay it. The success rate of the procedure is still very low, and research could surely improve it to the point where we might expect a 25 per cent chance of pregnancy following embryo transfer, which is the probability that a fertile woman has of conceiving in an ordinary menstrual cycle.

But all of these comments relate to the appendix. What of the book itself? It has little to add that is new, much that could have been discussed is missing, and several statements are factually incorrect. As for its good points, it is sensible to suggest that we should re-name in vitro fertilization "external fertilization", a term that is infinitely preferable to "test-tube baby", which has alas probably come to stay. Grobstein also has an interesting chapter on "Becoming a Person", in which he propounds the common-sense view that "neither life nor the human quality begins in any generation. Human life, like that of all species, descends without break from

G.D. Heathcote is at Broom's Barn Experimental Station, Higham, Bury St Edmunds, Suffolk.

generation to generation. It waxes and wanes in complexity but never begins de novo". But he has an ungainly style of writing: talking of human attributes, he says "As a group they span the biological, behavioral, and social realms, constituting a kind of slope between animal and human states along which our species has moved evolutionarily and continues to cycle generationally'

Although there is much discussion about "circumventing the oviduct", a valuable opportunity is missed by failing to compare this with "circumventing the penis"; the ethics of artificial insemination, whether by donor or husband's semen, have been widely discussed in recent decades. On the technical side, it is disappointing to find no mention of ultrasound, which is now widely used as a non-invasive way of monitoring follicular development. There is no discussion of interspecific fertilization, and yet use of denuded hamster oocytes to bioassay the fertilizing capacity and examine the karyotype of human spermatozoa has been an exciting recent technical advance seems to send an ethical the World Health Organiz

As for the mistakes, how rtunate to depict luteinizing hormon product of the posterior how naive to believe that in is often the result of a low s that merely increasing spel centrifuging a semen sample fertility. Recent evidence f ite clearly oocyte preparations shows that oligospermic men are infe le because of some basic defect that results in the ejaculation of small numbers of spermatozoa each of which has a significantly reduced fertilizing potential; concentrating the ejaculate is therefore a waste of time. And Edwards and Steptoe, who after all pioneered this whole field, will be interested to hear that the work was done at Oldham Hospital in Cambridge, England! Not a book to recommend.

R V Short is Director of the MRC Unit of Reproductive Biology, Edinburgh.

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### **Lessons for twentieth-century nutritionists**

John Rivers

Nutrition and Nutritional Diseases: The Evolution of Concepts. By Karl Y. Guggenheim. Pp.378. ISBN 0-669-03950-0. (Collamore Press, Massachusetts: 1981.) \$21.95.

ALTHOUGH this is an important book, it must be admitted at the outset that it is not a historian's history. Professional historians of science will no doubt be irritated by the brevity with which many subjects are necessarily treated and by the occasional lapses that suggest historical naivety (for example, calling Boyle's school "Eaton", or seeming to equate Pereira's training as an apothecary with pharmacy not medicine).

But whatever its reception by historians, nutritionists will I hope buy and read this book, for it is an important history of their discipline and their profession, and one that they should study. It is all the more valuable because Professor Guggenheim is not a trained historian, but a nutritionist who, after a lifetime of research into the practical problems of nutrition, has produced this scholarly work in his retirement.

The discipline of nutrition is an uneasy coalition. Its scientific roots are in aspects of physiology which had their heyday in the nineteenth and the first half of the twentieth centuries. Its justification is a wide spectrum of unresolved practical problems, from tooth decay to the world food problem. And nutritionists, it must be admitted, are people who, by and large, fail either to revive the science or solve the

problems to which it is addressed. As long as it is possible to imagine that, even if the next experiment won't save the starving masses at a stroke, it is a step in that direction, then there is an excuse for surfing along on the wave-front of the present. But as it increasingly appears that poverty not protein is the key to malnutrition, and that tooth decay can only be cured by drawing the teeth of the confectionery industry, the nutritionist increasingly needs to take stock and decide whether he is really necessary.

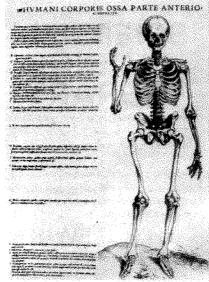
Individual action is of course irrelevant. What is needed is a dialogue within and beyond the profession about the extent to which nutritional science should be coupled to practical policy. Such a discussion would need to be guided by a coherent view of the past, and without the historian's spotlight can probably never begin. It is self-evident that such historical studies on nutrition as exist at present have not had any such catalytic role - not surprisingly, for where they are not social histories of diet, they are catalogues of achievements of the great and famous.

The significance of Professor Guggenheim's new book is that it is free of that tradition and hopefully will have a powerful impact. He does not merely retell the story of the development of the science of nutrition, but, more than any other work on the subject, he studies its growth mechanisms. His avowed aim is contained in the subtitle — he tries to trace "the evolution of concepts". He does so with great fluency, and in a way that is

realistic, in that it sees the concept as dominating the fact. Moreover, he makes his account relevant to today's problems by devoting a large part of the book to focusing "attention on the conceptual evolution of five principal issues that . . . dominate nutritional thinking until our own time". In doing so he has chosen well, and his discussions on the concept of an adequate diet, on protein and on the origins of the dietary thermogenesis controversy (recently revived in Nature) are all very valuable.

I am slightly disappointed by the fact that, inasmuch as he looks beyond internal imperatives for the growth of nutritional science, his horizon is the parallel growth of ideas in other areas of science. Highlighting this feedback is valuable, and he provides the best discussion of such interactions that is available in a popular work. But it is a pity that he did not extend his view and concentrate more on general social history and the extent to which nutritional science was also moulded by the general ethos of the society in which it was developed. In his failure to do so, his work raises questions it cannot answer. For example, his treatment of the evolution of concepts of protein and energy metabolism in the nineteenth century, while meticulous and well worth reading, is for me incomplete. I cannot believe that the subject can be fally explored without discussion of the social values that predicted it, stimulated both the research and its acceptance, and dictated its conceptual framework. Nutrition developed in the new Europe where both workhouse and standing army grew up, and the state required dietetic advice on subsistence requirements for the poor, and optimal provision for its fighting men.

It is surely no coincidence that in this environment nutritionists adopted models which involved the idea that both minimal



Skeleton of a rachitic male, depicted by A. Vesalius in Tabulae Anatomicae Sex (1538). Rickets was apparently so prevalent that Vesalius regarded the skeleton, of an adult with late rickets, as normal.



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social value of the social value of this book will encourage others to dit, perhaps, to provide some answers.

John Rivers is a Lecturer in the Department of Human Natrition at the London School of Hygiene and Tropical Medicine, University of London.

### Hectares for whom?

John Andrews

Farming and Wildlife. By Kenneth Mellanby. Pp.178. ISBN 0-00-219239-X. (Collins: 1981.) £9.50. Countryside Conservation: The Protection and Management of Amenity Ecosystems. By Bryn Green. Pp.249. ISBN hbk 0-04-719001-9; ISBN pbk 0-04-719002-7. (George Allen & Unwin: 1981.) Hbk £13, \$35; pbk £6.95, \$17.95.

SINCE prehistoric times, agriculture has been the major modifier of the world's terrestrial habitats: even the marine environment has been affected by the run-off of silt, organic nutrients and latterly of agrochemicals. In those countries where there is an active nature conservation lobby, relations with farming interests have become progressively more strained over the past 30 years as modern agricultural techniques have made rapid inroads into wildlife communities which had long coexisted with traditional farming methods. Inevitably, both sides have tended to misrepresent the situation to some degree and Professor Kenneth Mellanby's Farming and Wildlife provides a valuably calm and dispassionate appraisal of the effects of each on the other.

The opening chapters of the work deal in general terms with the impact of man on Britain's natural habitats and wildlife over the last 7,000 years, and more specifically with the effects of changing methods in arable farming, grassland management and livestock production since 1945. Greatly increased productivity from existing farmland has been won at the expense of a vast reduction in the population of until recently common and widespread wildlife, of which our native flowers and butterflies are perhaps the most regretted loss. At the same time, the profitability of farming and the incentives of grant aid have brought much marginal land into intensive production, severely reducing many species which were already rare. The middle section of the book looks in some detail at the features of typical lowland farms over which most public concern has been expressed — the soil itself; hedges and trees; and ponds, rivers and marshes.

Thus far the book contains much that will come as news to farmers who are often profoundly ignorant about the wildlife on the land and their own effects on it. By contrast, the final section contains much to inform the concerned but often equally ill-informed public about pests and pesticides, diseases of livestock and about field sports, which indirectly contribute to the retention of habitats and hence of wildlife in the countryside.

Farming and Wildlife is well illustrated and readable. No doubt conservationists will read it. The question is, will farmers?

Professor Mellanby deliberately eschews the political aspects of farming and wild-life, in particular the questions of whether increased agricultural output is economically justified and whether society should be able to control rural land use change where public loss of amenity is significant. However, these questions are examined with skill and relish by Bryn Green in Countryside Conservation.

It is timely that conservation, which has become an increasingly complex and skilled profession, now has its first text-book, and entirely apposite, given that the conservation movement is fighting desperately for what it believes in, that the text-book should also be a confident and well-argued assertion of its case.

Part 1 is devoted to conservation concepts, the impact of man on his environment and the development of conservation in Britain. In Part 2 the author examines the characteristics of natural ecosystems and explains the impact of land use change on all our major habitat types: agriculture figures largely, but forestry, industrial and urban development, recreational impact and pollution are given comparably thorough coverage. Conservation considerations aside, one is reminded again that land use management in Britain, where every hectare counts, is in appalling disarray, with blinkered state agencies each pursuing its own special interest regardless of the general good.

The concluding section of the book examines the present methods for selection and protection of land of conservation importance, and argues that a new approach is required if we are to save the remaining areas of scientific importance and the natural beauty of the countryside.

A welcome departure from the somewhat petulant "smocks and wind-mills" approach of some recent writers, Bryn Green's book contains much to instruct and to inspire a hard-headed approach to the massive problems which the conservation movement now faces.

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, Bedfordshire.

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Lecturer in Zoology at the University College of Wales, Aberystwyth.

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Biochemistry of Parasitic Helminths discusses the Biochemistry of Helminths in relation to that of mammals and also in relation to the biochemistry of free-living invertebrates. The book is divided into five sections. The first three parts cover biochemical components (emphasis being given to some of the more unusual compounds which occur in parasites), the pathways of energy production and biosynthetic reactions.

Part four covers, first, the ways in which metabolic pathways are controlled and inter-related. Second, it deals with the metabolic switches which occur during the life cycles of helminths and the ways in which these switches are under environmental and genetic control.

The last section gives a general summary of helminth biochemistry.

The text is intended primarily for use by specialist undergraduate courses in parasitiology and for research workers; it should also appeal to students interested in comparative and invertebrate biochemistry.

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### Why should anyone care about extinctions?

Kenneth Mellanby

Extinction: The Causes and Consequences of the Disappearance of Species. By Paul and Anne Ehrlich. Pp.305. ISBN 0-394-51312-6. (Random House, New York: 1981.) \$15.95. To be published in the UK in April 1982 by Gollancz.

PROFESSOR Paul Ehrlich is a distinguished entomologist, known for his meticulous studies of butterfly populations. He is also known to a wider public as a leading prophet of doom. Fortunately, when he has been rash enough to fix dates for particular disasters, his prophecies have seldom been fulfilled. Thus in an article published in 1969 he forecast that "there had been the final gasp of the whaling industry in 1973 . . . by 1977 the annual yield of fish from the sea was down to 30 million tons . . . by September 1979, all important life in the sea was extinct [and] Japan and China were faced with almost instant starvation . . . . ". Now marine pollution has had harmful effects, but the oceans are probably as productive today as they were 12 years ago.

Yet sensible men must agree that our species is increasing in number too rapidly, and that the world's resources are being used too wastefully. The horrors foretold by Ehrlich and others are still possible unless populations are controlled and resources safeguarded. However, I believe

that exaggerated statements of imminent disaster are generally counter-productive. Instead of acting as warnings, they actually induce the complacency they are trying to prevent. They have contributed to what another author has recently described as "a waning of public enthusiasm in America for environmental issues".

In this latest book, Paul and Anne Ehrlich are still concerned with problems of global survival, but they restrict their attention to the way in which man is eliminating many species of plants and animals which coexist with him on the surface of the Earth. They avoid any more rash forecasts of dates when extinctions will occur, and even suggest that we may have 15 or so years to put our house - our globe - in order. They do, however, fear that the rate at which species disappear will accelerate, particularly in the tropics where vast areas of forest are being felled. Few scientists will disagree with their general forecasts of the extinction of species, though their views on the effects on life on Earth are less generally acceptable.

Extinctions have always taken place. There is little doubt that the vast majority of species which have evolved during the 3,000 million or so years during which life has existed on Earth are extinct. Some have suggested that we should therefore not worry about further extinctions; they believe that new organisms suited to

today's world will evolve to fill the vacant niches. Unfortunately this is unlikely, because man produces such sudden changes that there is no time for new forms to evolve. Thus the Ehrlichs point out that much as we may regret, from an aesthetic and scientific standpoint, the passing of the dinosaurs, their place has been taken by mammals and birds. If today we kill off the remaining elephants and rhinoceroses, the world's fauna will be depleted before any replacements have appeared.

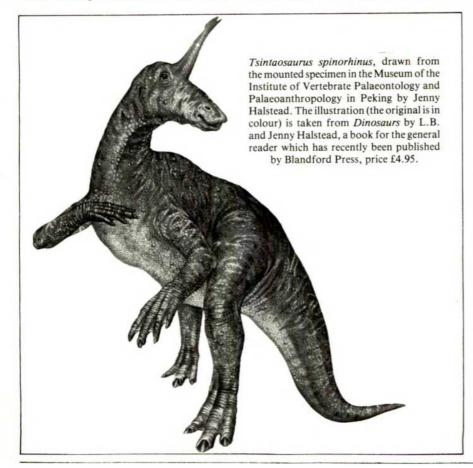
As a conservationist I regret all extinctions, whether locally as of the large blue butterfly in Britain, or globally, as of the dodo, the great auk or the passenger pigeon. I wish I could help to reverse the trend. But we must accept that the majority of people do not care, and in fact are doing much to accelerate the process. How can we convince them of the error of their ways?

The Ehrlichs give four reasons. First, compassion - all species on "Spaceship Earth" have an equal right to exist. Second, aesthetic and spiritual - wildlife contributes to the beauty and interest of the world. Third, economic - many organisms, their potential as yet unrecognized, may be exploited as food or other resources, or may provide essential genetic material for crops, livestock or drugs. Fourth, and in the Ehrlichs' view most importantly, by eliminating other species man is endangering the whole global life-support system, and so rendering our planet increasingly inhospitable to man himself.

What do we mean when we say that all species have an equal right to exist? Logically this would mean that man must do nothing to decrease their numbers or to reduce the area they inhabit. Even those who, for religious reasons, do not kill, farm the land; this can cause local or even general exterminations. If it is the species which has rights, then I suspect that we like to retain some members more for our purposes than for their own.

The Ehrlichs' second reason really boils down to saying that we wish to preserve the species which give us pleasure. This pleasure may have its spiritual or even its religious components, but it acknowledges that we conserve wildlife for man's satisfaction and not for the benefit of the wild creatures themselves.

The statement about the possible economic value of wildlife has some substance. Deer and antelope are proving more efficient converters of some types of vegetation than are cattle or sheep; there may be other animals which will be profitably domesticated. Man has been very conservative in his choice of food crops, and other wild plants may be valuable sources of food. Genes of wild plants may confer valuable properties to existing crop species, so wild grasses and Andean potatoes should be preserved. Unfortunately the species of wildlife for whose preservation most effort is being



made — the large carnivores and herbivores — would seem to have less potential except to provide pleasure and inspiration. But it is obviously good sense to try to preserve large areas of tropical forest and other habitats which contain thousands — perhaps millions — of undescribed species, whose economic potential has not been studied.

Finally, the Ehrlichs believe that unless we can maintain the diversity of the world's flora and fauna, the whole life-support system may break down. This is probably true, but the organisms which are the most important — the decomposers in the soil, insignificant parasitic insects, soil fungi and sewage organisms — are seldom the subjects of popular conservation programmes. The impoverishment of our fauna with the loss of the wolf, the bear and

the wild boar has *not* had the effect of making farming in Britain less efficient — quite the reverse.

So we must come to the conclusion that conservation is a far from simple subject. The economic and similar arguments often need to be qualified. Those based on the value of diversity are far from proven. Conservationists must avoid making statements which they know to be untrue in an attempt to obtain wider support from politicians and the general public. The most cogent reason for conservation, in my opinion, is that "man does not live by bread alone"; the general and aesthetic reasons have more validity than the economic.

Kenneth Mellanby's most recent book is Farming and Wildlife (Collins, 1981).

### Fighting for the conservation of tigers

Brian Bertram

Saving the Tiger. By Guy Mountfort. Pp.123. ISBN US 0-670-61999-X; ISBN UK 0-7181-1991-6. (Viking/Michael Joseph: 1981.) \$16.95, £7.95.

THE tiger is a slim, dramatic animal, and it now has a book to match it. The numerous, large and well-printed colour plates are spectacular and superb. They do not leave a great deal of space for the authoritative text, in which the author summarizes our still scanty information on tigers, their appalling conservation plight and international attempts to prevent their extinction.

Despite the title, the tiger has not been saved, and it would be dangerous to think that it had been. Equally, it would now be much closer to extinction but for international efforts on its behalf. Guy Mountfort was both the catalyst and the power house in setting up Operation Tiger in 1972, under which the World Wildlife Fund raised nearly a million pounds for tiger conservation. He describes how the governments of India, Bangladesh and Nepal were persuaded to establish reserves for tigers, and other countries of Southeast Asia have since followed suit.

The hunting of tigers for sport is no longer a threat, thanks to national legislation in most of the countries concerned. The traffic in tiger skins for the fur trade has dwindled, thanks also to CITES, the Convention on International Trade in Endangered Species. Illegal killing of tigers is declining as local policing improves and as there is less direct contact between tigers and the local populace. The main threat nowadays is from loss of the forest habitat and the prey species on which tigers depend. Through the efforts of Guy Mountfort and others, this threat has been alleviated in many areas, and general

public awareness of the plight of these wonderful animals has been raised.

What about future prospects? First, our priorities. With great difficulty and organization, large sums of money have been raised and thousands of square miles of tiger habitat protected. The total costs involved are equivalent to those needed to build a mere half-dozen miles of motorway. Which is more important? Should we have some form of conservation levy which because we human beings are so numerous would be paltry at the individual level?

Second, the status of tigers in their native reserves is relatively safe only so long as there is no breakdown of law and order in the countries concerned, and until human population pressures become irresistibly strong.

Third, although the many captive tigers in the zoos of the world are not a substitute for wild populations, they may well be a back-up. There should be tigers in the wild where they belong. But if the 350-400 wild Siberian tigers or the 600-800 wild Sumatran tigers are exterminated through human greed or folly, there are at least 800 Siberian and 150 Sumatran tigers in captivity. I am convinced that these could be used to re-stock areas from which wild tigers had been wiped out. Certainly, it would be difficult, and would need money, time and skill, and certainly there would be failures. But there is no reason in principle why, if necessary, reintroduction should not prove possible with tigers as it has with other large cats. We only hope that it does not become necessary. Guy Mountfort has done much more than most to help prevent

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### The sexes look at sex

Adrienne L. Zihlman

The Woman That Never Evolved. By Sarah Blaffer Hrdy. Pp.242. ISBN 0-674-95540-4. (Harvard University Press: 1981.) \$17.50, £12.25. The Evolution of Love. By Sydney L.W. Mellen. Pp.312. ISBN hbk 0-7167-1271-7; ISBN pbk 0-7167-1272-5. (W.H. Freeman: 1981.) Hbk \$15.95, £8.50; pbk \$8.95.

BOOKS on the evolution of human behaviour are often more interesting for the questions raised than for the answers proposed. This is particularly true when one compares the questions posed by male and female authors about the evolution of primate sexuality. The books under review provide a classic case study in contrasting, almost mutually exclusive, viewpoints.

During the 1960s, male reconstructions of human evolution informed us that human hairlessness evolved to enhance front-to-front sex; that men's social and political clubs have sprung from the cooperative cameraderie of the male hunting groups that made our species successful millions of years ago; and that human females lost oestrus and became perpetually sexually receptive so as to keep males around to provide protection and meat from the hunt.

During the 1970s, women increasingly joined men in the game of replaying human prehistory. In place of the prevalent Manthe-Hunter scenario that put males centre stage and relegated passive, dependent females to the wings, waiting for the heroes' return, women anthropologists emphasized the important social and economic role of Woman-the-Gatherer. They also drew on sociobiological theory, which hypothesizes that the sex investing the most time and energy in raising the offspring makes the choice of mates. The central male role in the evolution of human culture and institutions was disputed by field observations on primates and on gatherer-hunters such as the !Kung San, revealing the pre-eminent involvement of females in obtaining and sharing food, choosing mates, socializing children, establishing and maintaining social networks, and using tools - in contrast with the earlier image of females as rather passive recipients of male sexual and hunting activities.

Sydney Mellen's The Evolution of Love and Sarah Hrdy's The Woman That Never Evolved provide examples of the male and female viewpoints of the 1980s. Both are concerned with an evolutionary overview of human nature, particularly sexual nature. Both use the theoretical framework of sociobiology, which attempts to explain contemporary human behaviour by genes that evolved millions of years ago. Mellen draws heavily on the fossil and archaeological record of the past 2–3 million years and does not dwell on non-human

primates, whereas Hrdy goes deeply into the observed behaviour of females of numerous primate species and makes little reference to evolution as read from the fossil record.

According to Mellen, "love" emerged some three million years ago as an important adhesive force for human social groups and for successful child-rearing. His definition of love slides between conscious emotional attachment and sexual attraction; the large human brain is implicitly involved, but how human love differs from the emotional bonds of higher non-human primates is not always clear. His shortest chapter (7 pages) is titled "Love Between Parents and Children", the longest (46 pages), "The Enigma of Homosexual Love", by which he means male homosexual love.

Mellen's book belongs with the 1960s genre in that the framework is Man-the-Hunter and male choice of females. A central issue is male access to females, in order to be assured of their procreative potential, sexual receptiveness and "love" Hunting is credited with our acquisition of intelligence, cooperation, communication and food-sharing (old stuff); and (a new twist) men had many mates and natural selection enhanced "capacities for suspending fear" which resulted in the surviving hunters passing on "their courageous genes ... to many sons". Sociobiological theory tends to find a gene for every behaviour, and so Mellen explains that "finally the world came to be rather full of men whose chromosomes carried certain invidious genes for preferring young women", surely an unnecessary exegesis in a world in which, until very recently, few females or males lived beyond the age of 30 or 40.

The Woman That Never Evolved challenges some of the sexual stereotypes that Mellen perpetuates, almost to the point of reversing them. Hrdy argues that the ubiquitous domination of females by males is a product of primate evolutionary history, as male domination is the rule (with few exceptions) among non-human primates. She brings together a broad array of up-to-date information on females of many species, from galagos to gelada baboons. Of particular interest are the chapters on monogamous primates, among which females enjoy relatively privileged positions, and on those species such as lemurs and squirrel monkeys in which there is no clear dominance of males over females.

Hrdy lays the greatest emphasis on female competition and status-seeking, as though to prove that women have inherited all the same undesirable traits that men possess and therefore should be equally successful. She states that "competition among females is central to primate social organization", and that "every female is essentially a competitive strategizing creature". Obviously, all members of a group "compete" to some extent for food,

status and sexual partners, but, as in an atomic nucleus, the disruptive forces must be less than the cohesive ones if the group is to survive. In most species, male access to females is more limited than female access to males, hence male-male competition would be expected to be, and is observed to be, more intense than competition among females.

Hrdy's evolutionary construct is reminiscent of those of the 1960s, with a sort of reversal of roles. It is females rather than males who are competitive and statusseeking, but instead of forming clubs and political parties, they use their sexuality to gain power and domesticate males and keep them from killing their infants. Hrdy's observation that "outside" male langurs will sometimes take over a troop and kill the offspring of lactating mothers presumably so that they can reimpregnate these females with their own genes — is raised to the level of a paradigm: by the iron laws of sociobiology, primate males will try to "murder" offspring not their own and must be prevented from doing so by female strategems.

The woman that never evolved, according to Hrdy, is the woman invented by contemporary feminists: "created equal", having a natural sense of solidarity with other women and innocent of the male lust for power and status. Hrdy seems to assume that culture plays no role in the behaviour of women, and if certain kinds of behaviour are not found in other primates, they will not be found in humans.

I have improvised a dialogue between Mellen and Hrdy, to contrast their concerns and conclusions. Mellen: "Protohuman females were evolving in the direction of ever-increasing care and nurture of the young". Hrdy: "The vision of assertive, dominance-oriented females differs radically from existing stereotypes of female primates as non-stop mothers whose perennial preoccupation with nurturing offspring keeps them out of politics". Mellen doubts that pleasure from sexual intercourse is common for women. Hrdy retorts that female sexual activity is "assertive and temporarily insatiable".

Both of these books join the growing stacks of sociobiological attempts to integrate genes and human behaviour. They fail by ignoring the intervening levels that influence the outcome of behaviour development, socialization, symbols, ritual and values that are passed on nongenetically from generation to generation. Instead, they invoke hypothetical genes evolved either by hypothetical Pleistocene screenplays or deduced from selected observations on non-human primates and contemporary cultures. Mellen and Hrdy draw nearly opposite conclusions from their gene-based reconstructions. The book that has not yet been written is one that recognizes several levels of analysis, that integrates culture and biology and that interprets female and male behaviour in a mutually adaptive social system.

Adrienne L. Zihlman is a Professor of Anthropology at the University of California, Santa Cruz.

### Taste for travel and a naturalist's eye

A.J. Cain

The Roving Naturalist: Travel Letters of Theodosius Dobzhansky. Edited by Bentley Glass. Pp.327. ISBN 0-87169-139-6. (American Philosophical Society, Philadelphia: 1980.) \$8.

THEODOSIUS Dobzhansky was a truly remarkable man, of great personal experience, shrewdness, humanity and intellectual ability. He wrote clear, vigorous, masculine prose, expressing the forceful thoughts of a first-class observer. This book of his letters and reminiscences is a delight; one can dip into it anywhere and find oneself in grand company. Even when his reactions are the usual ones — to the grandeur of tropical forests, the horrors of temperate-zone poverty or "lousy bureaucracy" - he expresses them with a freshness that always precludes banality. The book would be excellent at one's bedside for those with strength enough to ration themselves to a single letter.

There is an excellent, short biographical

introduction by Bentley Glass which gives the major events in Dobzhansky's extraordinarily varied and interesting lifehistory. He was born in the Ukraine in 1900. In 1910 the family moved to Kiev. The boy, already a keen butterfly collector, went on a school excursion to the Caucasus; two years later he and a friend went off by themselves without parental permission to that fascinating region. His tastes for travel and entomology were already developed.

As a student at Kiev University, supporting his widowed mother, he had a difficult time towards the end of the Great War, and during the Revolution, in 1919, they experienced the Communist terror. Typhus, the invasion of the Polish Army, the death of his mother and severe privations in the winter of 1919–1920 were serious afflictions which he survived, becoming a private tutor, a graduate, an assistant to one of the professors of zoology and a tutor in the Workers'

Faculty, Soon after, he visited Moscow to see the genetical researches of Chetverikov and his group, who had stocks of Drosophila given to them by H.J. Muller. Then he migrated to Leningrad, and was sent on scientific expeditions to Central Asia to study genetic variation in domestic animals — he seems to have studied nearly every other biological phenomenon as well, especially human beings. The first part of this book is his enthralling reminiscences of his travels in Central Asia between 1925 and 1927. In 1927 he and his wife went to the USA to work with Thomas Hunt Morgan at Columbia University, and he began that illustrious career in population

genetics using Drosophila. The problems arising from his investigations into populations of Drosophila in the USA demanded wider genetical explorations. The letters in the book (unfortunately we are never told to whom they were written, which would often clarify their mode of treatment of some topics) were written on his visits to Brazil (1948-1953), to other countries of South America (1955-1958), to Israel, Lebanon and Egypt (1956) and India, Indonesia and New Guinea (1960). He must have been a superb correspondent. As is to be expected, since the reminiscences were taped later in his life and the first letter dates from 1948, his character, after the sometimes hair-raising experiences of his youth, was fully formed. Thus we see no development of character in the book any more than there is in, for example, the short stories of "Saki", but the absence of that is more than compensated for by being able to see through the eyes of so good an observer and writer such a variety of landscapes, organisms, people and situations, sometimes hilarious, often exasperating, but always exciting.

He had a naturalist's eye for animals, plants and people, and excellent appreciation of landscape (less so for geology), a sharpness for human (as well as animal) character, and a lovely dry humour, very like "Saki's", all of which come out just as well in his letters as they did in his conversation. No empty pomposity ever imposed on him, and not many other involuntary psychological deceptions. The freshness of his response to tropical nature seems at first naive (but never boring, gushing or silly) because it is so immediate, but naive he was not. He turned a sharp eye inwards on himself as well - there are some extremely interesting obiter dicta on his own reactions to mountains, uninhabited tropical islands, palms, the tropical night and the Holy

Dedicated drosophilists must be warned that there is little in the book about *Drosophila*. Everyone else will find a lot in it.

### Missing the essential Professor Eysenck

P.E. Bryant

Hans Eysenck: The Man and His Work. By H.B. Gibson. Pp.275. ISBN 0-7206-0566-0. (Peter Owen, London/Humanities Press, New Jersey: 1981.) £11.95, \$26.

THE mainspring of the considerable body of research carried out by Professor Eysenck and his colleagues has always been his theory of personality. This is based on two ideas: the first is that everyone's personality can be described in terms of the person's position along three separate dimensions, and the second that the mechanisms which underlie these dimensions are, in principle, discoverable.

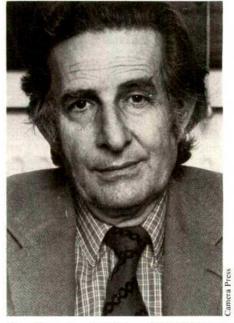
None of the specific details of his account of personality — the use of factor analysis, the three dimensions of introversion—extraversion, neuroticism and psychoticism or the use of the notions of excitation and inhibition and, later, of arousal to explain these dimensions — was in itself particularly new, but it has led to a great deal of original and fruitful research and it has also propelled Eysenck into a series of major controversies.

Anyone who has spent half-an-hour or more with one of his many books will know something of the theory and will also have learned that Eysenck not only revels in these controversies but also writes about them vividly and lucidly. Such characteristics are a distinct advantage in a psychologist. Controversy is the life-blood of psychology. The subject is still finding its feet, and this means that nothing much is certain and nothing can be taken for granted. Every single claim has to be argued over.

No one has recognized this or demonstrated it more than Eysenck. He is nettled, and often quite rightly, by anything that looks like a respectable consensus. It has been the established academic view that individual differences are rather unimportant in psychology, that people with extreme left-wing views are very different in personality from people on the extreme right, that psychoanalysis works, that a person's intellectual abilities are largely determined by the environment in which he grew up, that astrology is bunk and that smoking is a cause of cancer. Eysenck has at various times disagreed quite violently with all these respectable sentiments, and whether or not his objections are right they are always argued

His relish for battle and the clarity with which he presents his blow by blow accounts of them to psychologists and laymen alike represent an important contribution to psychology, but there is much more besides. He, more than anyone, shaped the development of clinical psychology in this country. He, too, fought valiantly and successfully against the unfortunate tendency among psychologists to

break up into quite separate camps which do not talk to each other and which pursue different questions with quite different methods. Eysenck insisted that the question of personality was too important to be left just to the personality testers. The methods of experimental psychology, he argued, were also needed to explain why people are different from each other. He managed to build a bridge between the two camps which has lasted extraordinarily well, so that nowadays no one using personality questionnaires can afford to



Hans Eysenck — "nettled . . . by anything that looks like a respectable consensus".

ignore laboratory research just as no one doing laboratory experiments can resort to the easy assumption that what is true about the behaviour of one person is true as well of everybody else. This link between two hitherto separate disciplines is in my view Eysenck's greatest achievement.

Such a man obviously deserves the accolade of a biography, and H.B. Gibson's account of Eysenck's life coming out as it does near to his retirement ought to give us a good idea not only of Eysenck's own development but also of his considerable influence on psychology over the last three decades. But it does not. Gibson's book is at its strongest when it recounts the simple facts about Eysenck's life - his early upbringing in the Weimar Republic and then under the Nazi regime, his revulsion against the Fascist system and his consequent move to England, his decision faute de mieux to study psychology (mieux in this case being physics), his early work with Burt and then his subsequent move under the tutelage of Aubrey Lewis to the Institute of Psychiatry in order to set up the clinical psychology department there.

But we need to know more than that and

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in particular we ought to be told in some detail about the controversies that have taken up so much of Eysenck's academic life, and also about the kind of man he is. On both these counts Gibson's book is a clear failure. He hardly deals with the controversies at all. Eysenck's own views are described reasonably well but the arguments about them, though sometimes mentioned, are never pursued, and again and again Gibson resorts to the excuse that the issues are too complex for his book. This is indeed an unfortunate contrast with the subject of his book who has never hesitated to introduce the layman to exactly the issues which Gibson so consistently avoids.

Thus one of the most obvious opportunities of a book like this — the chance to see how psychology has advanced with the help of lively debates seen through the life of one of its main controversialists - is almost totally lost. Worse still, at times the book's account of Eysenck's controversies is quite seriously misleading. For example, there is a chapter called the "Psychology of Politics" which deals with two very disparate topics. One is Eysenck's theory, published in the 1950s, of the personality types associated with various political views, and in particular his amusing claim that right- and left-wing extremists have much in common, a suggestion which did not endear him either to the left or to the right. The second is the debate about the question of the relative effects of heredity and environment which Eysenck formed so vigorously in the 1970s. As far as I can see, Gibson's only reason for putting these two issues together is his idea that one of the reasons for the violent hostility which greeted Eysenck's views about the importance of heredity was the vindictive rage felt by left-wing elements at the earlier suggestion that they were brothers under the skin of the Fascist foes. There may have been people as lunatic as this, but there were also some serious, well-argued objections which we are not given. We hear about the riots and the punches, but not about the debate.

Nor is the book much more helpful about Evsenck's own personality. It seems that over the years he fell out with many of his important colleagues, but the reasons why remain obscure. Eysenck is apparently shy: yet he has the habit of making outrageously immodest, public claims about his own abilities. Gibson has no explanation for this seeming paradox. Another issue which is left quite unsettled concerns Eysenck's attitude to other people. Gibson takes pains to show how kind Eysenck can be, and often the book adopts the style of a life of one of the saints as it ramblingly recounts this or that good deed by the professor. St Francis gave his cloak to a poor man: Eysenck took a whole day off to fetch a colleague's baby from hospital. But the book also relays some ungenerous comments made by Eysenck about and sometimes directly to other people. I simply do not know what to conclude, and

yet the question is important because one of the major reasons for Eysenck's success has been his ability to keep a large number of people around him working with enthusiasm on his ideas. I should have liked to have known how he did it.

Gibson's book is interesting and often

entertaining. But it gives us nothing like a proper assessment of Eysenck's formidable contribution to psychology over the past three decades.

P.E. Bryant is Watts Professor of Psychology at the University of Oxford.

### Archaeology in retrospect and in prospect

### J. Desmond Clark

A Short History of Archaeology. By Glyn Daniel. Pp.232. ISBN 0-500-05041-4. (Thames and Hudson: 1981.) £9.50, \$17.95. Antiquity and Man: Essays in Honour of Glyn Daniel. Edited by John D. Evans, Barry Cunliffe and Colin Renfrew. Pp.256. ISBN 0-500-05040-6. (Thames and Hudson: 1981.) £25.

It is probably true to say that no school of archaeology has done more in the decades immediately preceding and following the Second World War than has that at the University of Cambridge. There are the unique contributions of Grahame Clark to understanding Mesolithic and Neolithic economy; of Charles McBurney to Palaeolithic studies through his excavations in North Africa, Iran, Afghanistan and Britain; of David Clarke in revolutionizing theory and concepts in archaeology; and of Glyn Daniel through his special interest in and encyclopaedic knowledge of the history of archaeology and the study of megalithic monuments.

These were scholars unsurpassed in their fields and their experience, knowledge and teaching have been responsible for the training of an unrivalled nucleus of the leading archaeologists working in the Western world today. In part this stems from the alliance between archaeology and anthropology that has always been present at Cambridge and the success of archaeological interpretation and model building has come from the understanding of human behaviour provided by ethnographic studies, not infrequently now being undertaken by the archaeologists themselves. While archaeology derives much of its methodology from the natural and earth sciences, interpretation can only come from the insights of the anthropologist. When Professor Glyn Daniel retired this July after 8 years in the Disney Chair at Cambridge and 35 years on the faculty, an era in British archaeology drew to a close. A new group of scholars, often his own students, have taken up the challenge of the modern, conceptual approach to the discipline and are among the leading contributors to it.

There is no one who has made more of an impact than Professor Daniel on our understanding of the history of archaeology and the way that this has influenced current theory, and on the story of its emer-

gence from the enveloping strait-jacket of the Book of Genesis to become the creative and exciting discipline it is today. To celebrate his general editorship and the appearance of the hundredth volume in the Ancient Peoples and Places series published by Thames and Hudson, he has produced A Short History of Archaeology.

The volume is divided into five main chapters that cover the growth of archaeological method and theory from the beginnings, through the formative and then the developmental years between the wars, to that of the "new and not-so-new archaeology". This is a well-written, witty and enjoyable summary of the main conceptual, analytical and methodological advances in the field of prehistoric and historic archaeology as manifested by accounts of the increasing numbers of significant and often very exciting finds, the development of survey, excavation and recording techniques, and the awakening interest of the general public. In other words, it is an excellent history of mankind's ideas about his ancient past. This approach enables archaeologists to appreciate the value of the historical framework that has made possible the advances in field work, analysis and interpretation of the past two decades. By trying to understand the tenor of intellectual thought at a particular time we are better able to appreciate the major developments in archaeological theory and the reasoning that lies behind them.

Following the ordering of assemblages of artefacts in the Three Ages System, to that of the Stage or Age based on stratigraphic excavation and the concept of the "type fossil", archaeology has moved on to determining patterns of economic and social behaviour. The major concern with chronology and time-depth, which for so long occupied the earlier archaeologists, has only been removed since the availability of techniques such as radiocarbon, potassium-argon and the palaeomagnetic reversal chronology made possible by physicists, chemists and geologists since 1950. We are now, therefore, in a position to know, even if we cannot comprehend, the magnitude of the time involved in the story of our biological and cultural evolution.

A Short History of Archaeology is a synthesis not only of the record for

Western Europe but sets out to cover most of the world where archaeologists have made significant contributions. Thus the history of archaeology in the Americas, Africa, the Middle and Far East, and Australasia is discussed, though more satisfactorily for some areas than for others, depending on the author's viewpoint. The dramatic discoveries of Layard, Sohliemann, Petrie, Howard Carter, Arthur Evans and a host of others help to show the way in which archaeology has advanced from an over-emphasis on artefact morphology and typologies to the attempt to learn about the behaviour and the individuals that lie behind the tools themselves. The contributions of the systematic excavators and recorders - Pitt Rivers, Petrie, Mortimer Wheeler - and of those who sought to understand the processes behind culture change like Gordon Childe and the ethnographers receive here the credit they deserve.

Only in the last section of the book on the "new archaeology" does one get the impression that the author is not really in sympathy with his subject. Even though there is not all that much which is new in the more theoretical frameworks and the problem-orientated archaeology of today, it is the emphasis on this problem-orientated approach, coupled with the great advances in technique and method such as "edge polish" studies, spatial analysis, taphonomy and experimental archaeology, that is bringing about such an important re-orientation in our attitudes.

One thing that stands out from Glyn Daniel's intensive search of the literature is the way in which the intellectual world becomes receptive to new hypotheses and new constructs only when it is ready to do so and, although others before may have made the same observations and drawn the same conclusions, unless the scientific world is waiting for them they are quickly forgotten. The manner in which preconceptions can retard true understanding of the evidence is well seen in the early interpretation of Neanderthal man and the ease with which the Piltdown hoax was perpetrated. Glyn Daniel has led the way in showing that archaeology can be of interest for the layman as for the specialist and his works have been the forerunners of those hautes vulgarisations - accounts of all those prehistoric discoveries and palaeoanthropological wranglings - that delight the public today.

An excellent, well-selected set of colour and black and white illustrations accompany the text and this will certainly be a standard introductory textbook for years to come.

Antiquity and Man is not the usual kind of Festschrift which, all too often — as Glyn Daniel himself has said — is "a cemetery of articles which ought to have been published elsewhere in more accessible form, or not at all". The Prince of Wales, who took the archaeology Tripos Part I at Cambridge and was taught by

Glyn Daniel, contributes a foreword, and essays by some 28 leading authorities on archaeological topics and other matters make up the volume. The subjects treated are of direct interest to Glyn Daniel who has had a major involvement with and made a great impact on most of them. It is both a stock-taking of the state of archaeology in the continents today and a discussion of some of the leading theories that lie behind recent advances.



Glyn Daniel, who retired earlier this year after 35 years at Cambridge.

The book is divided into four unequal parts and comprises a valuable series of summary papers on a range of topics which show the extent of Professor Daniel's interest in and contributions to the discipline and also to Cambridge with its college dons and undergraduates. Space does not permit - and it would be invidious to single out - individual contributions from this outstanding text but the first part contains several excellent summaries of the current state of archaeology in Europe and the Middle East, Africa, Australia and China. The reader is made to appreciate the contribution of the Cambridge School to the changing face of archaeology today in Third World countries — the need for the training of nationals to take over from the expatriates and the advantage for both of collaboration and the multidisciplinary, international team approach. Several authors make a plea for fieldwork and warn against its neglect in favour of the theoretical approach which is sometimes only too evident today. Fieldwork is indeed the life blood of archaeology, and models and hypotheses are worthless without the factual empirical data that come from meticulous field methods and objective analysis and interpretation.

Part II comprises nine essays on current theories concerning the construction of megaliths found from the central Mediterranean to Britain and Scandinavia. Glyn Daniel played no small part in elucidating the mystery of their origins and significance. This section will be of especial interest to European archaeologists and shows well the shift in emphasis from the monuments themselves to attempting to learn about the populations that built them and the different economic and social organizations that gave rise to these ritual centres, often associated with communal burial. The diffusionist theory for the spread of megaliths has now been largely replaced by one of local, autochthonous development, while the radiocarbon and TL ages now available show that, far from being derived from the eastern Mediterranean, the megalithic complex preceded in its inception the major monuments of the Ancient Near East.

Part III sets out Glyn Daniel's influence in many different ways in awakening and stimulating public interest in archaeology: as an editor, particularly of the Ancient Peoples and Places series, as an "anchor man" of the television programme Animal, Vegetable, Mineral with Sir Mortimer Wheeler and later of the Buried Treasure series with Paul Johnston. His influence could not have been greater than through his editorship of Antiquity, so ably helped by his wife Ruth as production manager. This is and will remain, it is hoped, one of the leading archaeological journals.

These essays also bring out, especially in Part IV, what is perhaps Professor Daniel's most significant contribution, namely his great success as a teacher. Many of the essays are written by his former students and the high praise and affection with which they all speak of him bears ample witness to the wit and erudition with which he enlivened his lectures and to his natural ability to produce enthusiastic and professional archaeologists who have remained his friends.

Besides being a valuable supplement to A Short History of Archaeology, this volume is full of entertaining asides about archaeology and publishing, amateurs in archaeology and the public image of the archaeologist. It also contains interesting information about Glyn himself, his many achievements and his progresses through the menus gastronomiques of Brittany and the wine country of the Dordogne in his pursuit of archaeology.

Both books show very clearly how the discipline of archaeology is a product of Western European civilization. But the focus has now shifted to the Third World and other countries since it is here that we are learning what it was that made us human. It is here also that archaeology has a greater role to play than in the Western world since, for many nations, it is the most important source of knowledge of their past. Even though this may be regarded as only small beer and lentils, the record is as good as or surpasses that which has gone before

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### ARTICLES

## Superluminal quasar 3C179 with double radio lobes

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VLBI observations of the quasar 3C179 reveal that its two milli arc second components have an apparent relative velocity of 7.6 times the velocity of light. This is the fifth radio source in which 'superluminal' motion has been reported but the first which also exhibits double lobe structure on the arc second scale. Statistical arguments which apply to such sources cause difficulties for explanations based on motion in a relativistic jet.

SUPERLUMINAL motion (apparent relative velocity, v > twice the speed of light, 2c) has been reported for components in the milli arc second radio structure of the strong radio sources 3C345, 3C273, 3C279 and 3C120. The phenomenon has been widely discussed and reviewed<sup>1-7</sup> and some competing, ingenious models have been proposed to explain the illusion 8.9. I report here a fifth example of the phenomenon. VLBI (very long baseline interferometer) observations of the 18 mag quasar 3C179 reveal an expansion rate between its two milli arc second components of 7.6c over the course of 1.2 yr. The source has a higher redshift (z = 0.843, see ref. 10) than the other four superluminal sources and the core is a factor of 10-20 weaker. Unlike the other superluminal sources it has classical doublelobe morphology on an arc second scale. This work was motivated by the suggestion by Scheuer and Readhead11 (repeated by Hine and Scheuer<sup>12</sup>) that such sources should not exhibit superluminal motions.

3C179 (= 0723 + 67) was selected from the quasars mapped by Owen, Porcas and Neff<sup>13</sup> using the NRAO Green Bank interferometer at 2.7 and 8.1 GHz. Thirty of these sources form a set with flux densities at 966 MHz ≥ 0.7 Jy, magnitudes brighter than 19 and angular sizes >10 arc s. Nearly all these quasars have detectable, compact central components. Amongst these, 3C179 has the strongest central component of ~0.5 Jy, contributing ~30% of the flux density at 2.7 GHz, and an outer double-lobed structure with a separation of 14 arc s in position angle (PA) ~80°. At frequencies below ~2 GHz the radio spectrum has a spectral index,  $\alpha$ , of  $-0.75~(S(\nu) \propto \nu^{\alpha})$  but at higher frequencies the spectrum flattens, indicating the presence of the compact component. The radio spectrum, derived from a compilation of published flux density measurements and recent observations, is shown in Fig. 1. The source was not known to be variable in flux density at the time of selection but comparisons of recent measurements at 5 and 10.6 GHz with measurements made in the late 1960s<sup>14,15</sup> suggest that the core is variable, both on a 10-yr and a 1-yr time scale.

#### **Observations**

VLBI observations were made of 3C179 at two epochs, 1-2 October 1979 and 9 December 1980, at a frequency of 10.7 GHz. The telescopes at Effelsberg (FRG), Haystack (Massachusetts, USA), Green Bank (West Virginia, USA) and Owens Valley (California, USA) were used, and the hydrogen maser frequency standards used at all the sites permitted coherent integration times of 4 min. The Mark 2 VLBI recording system used for these observations achieved an r.m.s. error per point of 12-20 mJy on the high resolution transatlantic baselines. The interferometer lobe spacing on the longest baseline is  $\sim 0.5$  m arc s. In both observing sessions at least 11 h of good data were obtained at all the telescopes. The data were processed using the Max-Planck-Institute VLBI processor in Bonn and the visibility functions were calibrated using

measurements of the individual antenna gains and system temperatures.

#### Results

The most interesting structure in 3C179 is revealed by the high resolution transatlantic baselines. In Fig. 2a, c, e, the visibility functions measured on these baselines in October 1979 are plotted. These clearly show the oscillatory nature characteristic of a double morphology. Simple gaussian modelling of the sky brightness distribution gives an excellent fit to the visibilities with two components, of flux ratio ~2:1, separated by 1.07 m arc s in PA  $92^{\circ}$ . This orientation is within  $\sim 12^{\circ}$  of the PA of the outer structure although the latter is not well defined as both outer components are heavily resolved13. This may be compared with the good alignment of inner to outer structure generally observed in the central components of double-lobed sources such as Cygnus A, 3C111, 3C390.3, NGC6251 and 3C236 (refs 16-19). However, this is the first time that the structure of the central component of a double source identified with a quasar has been determined. Those quasars which have been observed to date have asymmetric outer structure and generally show marked bending between the angles of the inner and outer structure (for example, 3C380, 3C147 and the superluminal sources 3C345, 3C273 (ref. 19)).

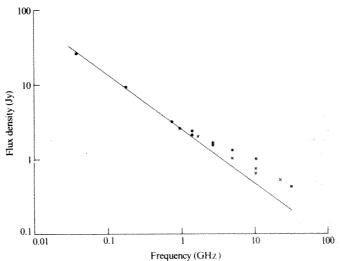


Fig. 1 The radio spectrum of 3C179. Reference numbers for measured flux densities at different frequencies are shown in parentheses. UE, unpublished measurements made with Effelsberg 100-m telescope between 1979 and 1981, marked with crosses. 38 MHz (35); 178 MHz (36); 750 MHz (37); 966 MHz (10); 1.4 GHz (37, 38); 1.7 GHz (UE); 2.7 GHz (13, 39); 5.0 GHz (14, UE); 10.7 GHz (15, UE); 22.7 GHz (UE); 31.4 GHz (40). The straight line through the low frequency points represents a spectral index of -0.75.

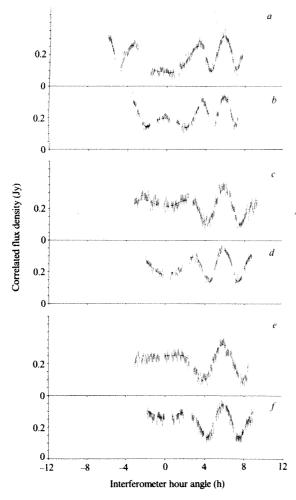


Fig. 2 Correlated flux density of 3C179 on various baselines versus interferometer hour angle. The solid line represents the best two-component fit. Baselines: a, b, Effelsberg-Owens Valley; c, d Effelsberg-Green Bank; e, f, Effelsberg-Haystack. a, c, e were measured in October 1979; b, d, f were measured in December 1980.

The visibility functions for the same baselines observed in December 1980 are shown in Fig. 2b, d, f. Again the structure is clearly double, although it is equally apparent that structural changes have occurred. The most interesting feature is the narrowing of the distance between the peaks and troughs of the visibility function, corresponding to a larger separation of the two components at the later epoch. Gaussian model fitting confirms this, giving a separation of 1.24 m arc s in the same position angle. The results of the gaussian parameter fits to the data from both epochs are given in Table 1, along with the single dish flux densities for the whole source measured in Effelsberg. With the standard values of the cosmological parameters used in previous studies of superluminal sources  $(H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}, q_0 = 0.05) 3C179 \text{ has an apparent separation}$ velocity between components of  $7.6c \pm 1c$ . This makes it the fifth known source exhibiting superluminal motion between milli arc second components. Projecting back the separation rate gives the epoch of zero separation as ~1972.0; unfortunately, no flux density monitoring of 3C179, which would indicate any outbursts, seems to be available for this time.

An additional change that has occurred during the 1.2 yr between the two observing epochs is the depth of the minima in the visibility functions, which have become shallower with time, indicating that the flux densities of the two components have become more unequal. This is very similar to the behaviour of the superluminal source 3C345 reported by Cohen et al.<sup>20</sup>; however, in the case of 3C179, the model parameters and total flux densities given in Table 1 indicate that the stronger component has increased, whereas in 3C345 it is the weaker component which decreases or perhaps becomes more diffuse and complex<sup>20,21</sup>.

At both epochs the individual components each seem to be resolved and it is unclear from the models which of the components could be identified with the core and the jet usually mentioned in the context of radio source milli arc second structure. There are strong indications from the closure phase, however, that the stronger (east) component is more compact than the weaker (west) component, and thus corresponds to the core.

Pearson et al.<sup>7</sup>'s hybrid maps of the strong source 3C273 clearly demonstrate the superluminal motion of one of its components. Although this technique is unsuitable for the present observations of 3C179 because of the relatively poor sensitivity on many of the inter-American baselines, the following checks indicate that the gaussian model well represents the real source structure:

- (1) The r.m.s. residuals from the model fit are in close agreement with those expected due to random noise and small  $(\sim 3\%)$  calibration uncertainties.
- (2) Attempts to force a fit at either epoch with the mean angular separation of 1.16 m arc s, and allowing component sizes and fluxes to change, fail to produce a good fit to the data.
- sizes and fluxes to change, fail to produce a good fit to the data.
  (3) The closure phase<sup>22</sup>, where it exists, is in excellent agreement with that predicted from the models.
- (4) Separately model fitting the individual baselines independently gives the separations quoted in Table 1 at each epoch.

#### Discussion

It is interesting to compare the observed behaviour of 3C179 with the various models previously proposed to account for the phenomenon. However, many of the observational tests for distinguishing between models require more than two observing epochs. For 3C179 the two epochs indicate a superluminal expansion rather than contraction and it is the fifth source where this is true. (Random brightening of stationary components, as proposed by Dent<sup>23</sup>, would predict an equal number of contractions). The magnetic dipole model<sup>24</sup> requires a separation velocity >4.4c; on the basis of the cosmological parameters adopted, this condition is satisfied by the present observations.

Two further explanations of the superluminal effect require intrinsically improbable circumstances which, however, are preferentially selected when compact sources with strong radio emission are observed. In the gravitational lens model<sup>25</sup> a foreground galaxy magnifies a subluminal motion in the quasar; angular magnification makes the motion superluminal and flux density magnification makes the source bright. The compact core in 3C179 is much weaker than those of the previously known superluminal sources and thus the preferential selection

			Table 1	Model parameters				نه ،
Epoch	Single dish flux (mJy)	Component	Flux (mJy)	Separation (m arc s)	PA (deg)	Major axis size (m arc s)	Minor axis Major axis	PA (deg)
October 1979	$670 \pm 15$	W E	$114 \pm 5$ $209 \pm 8$	- 1.07 ± 0.01	92±1	$0.34 \pm 0.05$ $0.24 \pm 0.05$	$0.2 \pm 0.1$ $0.0 \pm 0.1$	$95 \pm 5 \\ 88 \pm 5$
December 1980	$720 \pm 15$	W E	$107 \pm 5$ $240 \pm 8$	$1.24 \pm 0.01$	92±1	$0.32 \pm 0.05$ $0.18 \pm 0.05$	$0.3 \pm 0.1$ $0.0 \pm 0.1$	82±5 78±5

cannot be operating so strongly, unless central components of double-lobed sources are intrinsically weaker.

The most widely discussed explanation involves relativistic motion of the radio emitting regions in the superluminal sources. In the relativistic jet model, for example 11.26, the superluminal effect arises from the time delay between the two components when the angle,  $\theta$ , of the motion to the line of sight is small. The relativistic motion towards the observer has the additional effect of enhancing the measured flux density by relativistic Doppler boosting (see ref. 27). This model has two essential elements: the Lorentz factor,  $\gamma$ , of the relativistic motion must be  $\geq$  the apparent velocity/c and for the minimum value of  $\gamma$  (in this case  $\gamma = 7.6$ ) the angle,  $\theta$ , to the line of sight must be  $\sim 1/\gamma$  ( $\theta \approx 7.5^{\circ}$ ).

As there seems to be good alignment between the position angle of the structure of central components and that of the outer lobes, one might assume that the entire source axis is inclined at this small angle, making the true, deprojected angular size  $\sim 107$  arc s. This is rather large for a quasar at this redshift (see ref. 28) and corresponds to a linear size of ~1 Mpc; this is not, however, compelling evidence against such a small angle.

A more serious objection arises from the enhancement of flux density associated with high  $\gamma$  and small  $\theta$ . 3C179 was chosen from a sample of 30 quasars with large (≥10 arcs) structure. Although the selection of 3C179 from the sample is biased towards a high  $\gamma$  and small  $\theta$ , the sample of 30 sources is not biased towards small  $\theta$ , because the outer lobe flux densities, which dominate in these sources, have been shown to be 'unbeamed'<sup>29,30</sup>. Thus it is unlikely, a priori, that a source with the required small angle  $(P \sim 0.009)$  exists in the sample with the

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required value of  $\gamma$  unless  $\gamma \sim 7.6$  is typical for the sample. (This argument is independent of any alignment of inner and outer structures). Scheuer and Readhead's prediction of a lack of super uninal motion in such sources is based on their assertion that the typical  $\gamma$  can be no more than  $\sim$ 2 in such double sources because the observed range of boosting factors in central components is low. If this argument is correct, 3C179 must be regarded as a freak within the sample, with  $\gamma$  and  $\theta$  conveniently contrived for the observation of the superluminal

Because relativistic motion has been used to explain the rapid flux density variations in compact sources<sup>31</sup>, and relativistic jets ecount for the efficient transfer of energy from the nucleus to the outer lobes (see ref. 32) and other morphological aspects of radio sources<sup>33,34</sup>, there are reasons for retaining the idea of relativistic motions in compact radio sources. One way to ease the problem is to adopt a rather higher value of the Hubble constant. With  $H_0 = 100$  the required  $\gamma$  becomes  $\sim 4.2$ ,  $\theta \sim 14^\circ$ and overall size ~300 kpc for 3C179. However, the detection of superluminal motion in the first source of the type predicted not to how it suggests that the effect may be very much more common than at present thought.

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### Non-tandem arrangement and divergent transcription of chicken histone genes

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We have located chicken histone genes within 14-kilobase pd inserts of two genomal clones, λCH-01 and λCH-02. The genes were mapped with chicken histone cDNA and gene-pecific probes, and the identity and polarity of each gene confirmed by DNA sequencing. As previously indicated, histolee mRNAs are transcribed from both DNA strands. The gene organization differs in the two clones. Although there is some clustering of genes, no repeating unit is observed. λCH-01 contains all core histone genes, but two copies of H2A occul before the full complement of histone genes is represented. λCH-02 contains no H4 or H2A genes, but notably has two adjacent H3 genes which are divergently transcribed. H2A, H2B and H3 genes all appear more than once in the combiled inserts of the two clones, but in no case do they occur in equivalent environments. This indicates a considerable degite of disorder of the histone genes in the chicken genome.

HISTONES comprise a small group of basic proteins whose primary function is in forming the structural matrix of nucleosomes (for review see ref. 1). The amino acid sequences of histones exhibit only a narrow spectrum of divergence through

evolutionary time, consistent with their structural role in the ubiquitous nucleosome particle. In addition, species- and tissuespecific histone subtypes have been widely observed and these are regulated during embyrogenesis and spermatogenesis

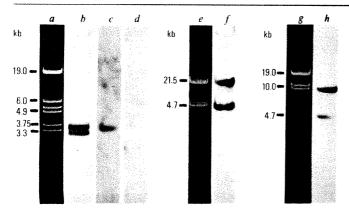


Fig. 1 Hybridization of 'histone' cDNA and specific gene probes to λCH-01 and λCH-02. Restriction digests of λCH-01 and λCH-02 DNA were electrophoresed in 1% agarose slab gels and the DNA transferred to nitrocellulose sheets<sup>28,29</sup>. The filters in tracks c, d, e and f were hybridized with <sup>32</sup>P-DNA probes labelled by nick translation<sup>30,32</sup>P-cDNA (tracks b, h) was reverse-transcribed from 5-day embryo 7-11S RNA<sup>17</sup>, using the random priming method<sup>31</sup>. The sea urchin H3 and H4 probes were constructed from fragments of the sea urchin H3 and H4 probes were constructed from fragments of the sea urchin H3 and H4 probes were constructed from fragments of the sea urchin H3 and Fagment of the H2B gene, track d shows that this does not hybridize to the chicken H2B gene. The chicken H2A probe was the Xhol-Xhol fragment of pCH3.3E (see Fig. 2a, fragment D), resolved on polyacrylamide slab gels and eluted as for DNA sequencing<sup>33</sup>. a, A Hind3-EcoRI digest of λCH-01 viewed by UV illumination of an ethidium bromide-stained gel. Sizes were determined by reference to Hind3-digested λ DNA run in the same gel. b, c, d, Hybridization to this λCH-01 digest with histone cDNA, sea urchin H4 probe and sea urchin H3 probe, respectively. e, A Sma-Hind3 digest of λCH-01. f, Hybridization to this digest with the chicken H2A probe. g, An EcoRI digest of λCH-02 visualized ethidium bromide-stained fragments. h, Hybridization of this digest with histone cDNA.

(reviewed in ref. 2). The amino acid changes which specify each subtype often occur in the otherwise conserved globular domains. These changes, and a variety of post-synthetic modifications<sup>2,3</sup>, may alter nucleosome structure and function. It has been suggested that subtype switching is intimately related to chromatin remodelling during development of new cell types<sup>4,5</sup>. The interaction of histones with both cell cycle and developmental events implies that their expressional requirements may be complex. Superimposed on this, the structure of the nucleosome dictates the need for strictly equimolar amounts of 'core' histones, and approximately half molar amounts of the

H1 group<sup>6</sup>. These requirements have provoked much interest in the structure and evolution of histone genes.

The highly reiterated 'early' histone genes from several species of sea urchin have been extensively characterized (for review see ref. 7). A repeating unit containing one each of the five histone genes is reiterated in tandem along the chromosome. Some histone variants are encoded in the DNA of less frequent repeats<sup>8</sup>. Selective expression of different repeat units has been observed, accounting for the appearance of variant histone proteins at specific stages of sea urchin development<sup>9,10</sup>. It has been recently shown that individual histone gene 'orphons' are also present in sea urchin DNA<sup>11</sup>.

The histone genes of *Drosophila* are organized into a compact repeat unit containing all five genes<sup>12</sup>, but both gene order and relative gene polarity differ from the sea urchin arrangement. In *Xenopus*, histone genes are clustered and tandem arrangement is observed<sup>13,14</sup>. Nevertheless, preliminary examination of two clones shows that gene order may vary<sup>13,14</sup>. In yeast<sup>15</sup> the genes for the H2A and H2B proteins are linked and transcribed in a divergent fashion. They are represented only twice in the yeast haploid genome and the two H2A/H2B pairs are spatially distinct from one another and also from the H3 and H4 histone genes.

We have previously reported the isolation of a genomic chicken histone clone,  $\lambda \text{CH-}01^{16}$ . Here, we locate and orient core histone genes in this clone and extend the analysis to a non-overlapping clone  $\lambda \text{CH-}02$ . There is no indication of tandemly repeated genes in these inserts, and the data indicate a lack of order of chicken histone genes.

#### Coding potential of $\lambda$ CH-01 and $\lambda$ CH-02

Coding regions were located in each unit by hybridization of restriction digests of the whole clone with 'histone' cDNA, and all cDNA-positive regions were subsequently shown to contain core histone-coding regions by DNA sequence analysis. Although the embryonic RNA from which the cDNA was transcribed translates into the five histone proteins in the wheatgerm cell-free system<sup>17</sup>, it is possible that embryonic cDNA does not detect tissue-specific H1 gene variants<sup>13</sup>.

Two major coding regions were identified within a Hind3-EcoRI digest of  $\lambda CH-01$  (Fig. 1a, b and ref. 16). These two fragments of 3.3 and 3.75 kilobase pairs (kbp) were ligated into the plasmid vector pBR325 to generate the subclones pCH3.3E

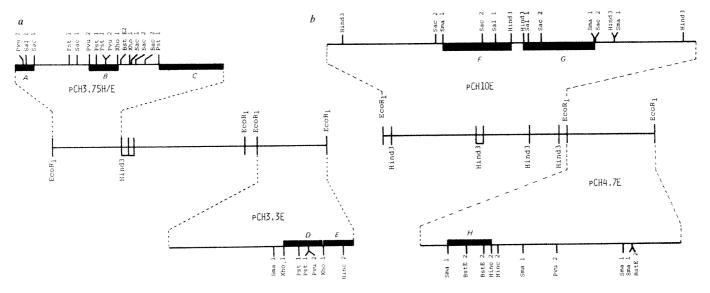


Fig. 2 Restriction endonuclease maps of λCH-01, λCH-02 and subclones derived from them. Blocked-in regions indicate fragments which hybridized with histone cDNA. Combinations of restriction enzyme digests were used to compile the data from the plasmid subclones and only those sites are shown which were of practical use in precise location and subsequent DNA sequence analysis of individual genes (see Figs 3-5). The insert size of λCH-01 is 14 kbp and that of λCH-02 14.7 kbp. All maps of the subclones except pCH10E have been enlarged three times relative to the overall λCH-01 or λCH-02 maps. The size of pCH10E has doubled relative to λCH-02. The position of the small EcoRI fragment of λCH-01 is corrected here from its previous allocation 16. a, cDNA-positive regions A, B, C, D, E of λCH-01 subclones pCH3.75H/E and pCH3.3E, respectively. b, cDNA-positive regions F, G, H of λCH-02 subclones pCH10E and pCH4.7E, respectively. These regions are referred to in the text and are subsequently identified by DNA sequence analysis (Figs 3, 4).

and pCH3.75H/E, respectively. The subclones were mapped with restriction endonucleases and coding regions were located in finer detail using histone cDNA (Fig. 2a). Five coding regions (A-E) were identified, suggesting that  $\lambda$ CH-01 encoded multiple genes.

A similar analysis of  $\lambda$ CH-02 revealed coding regions within both the 10- and 4.7-kbp EcoRI fragments (Fig. 1g, h). These were subcloned (pCH10E and pCH4.7E) and further mapping and cDNA hybridization studies revealed three coding regions, F-H (Fig. 2b).

### Detection of genes in CH-01 using specific coding probes

To identify the coding regions indicated in Fig. 2a, we screened  $\lambda$ CH-01 with specific gene probes. Due to the conservation of histone amino acid sequences through evolution, sea urchin probes have proved useful for detecting histone genes of other species<sup>12</sup>. Our own findings suggest, however, that these cross-species probes must be used with caution. Although the H4 sea urchin probe showed specific hybridization to chicken recombinants, the sea urchin H3 probe and a probe containing a fragment of the *Drosophila* H2B gene gave ambiguous hybridization results when used on  $\lambda$ CH-01. Similarly, results from this laboratory indicate that the sea urchin H3 gene probe does not cross-hybridize unambiguously with an authentic human H3 histone gene. These observations question the results of previous attempts to detect histone gene transcripts in mammalian cells<sup>18,19</sup> using sea urchin probes.

The presence of an H2A gene in  $\lambda$ CH-01 has been previously recognized from DNA sequencing <sup>16</sup>. This was unambiguously located in pCH3.3E (Fig. 2a, region D) from the position of restriction sites. An H2A gene-containing fragment bounded by XhoI sites (Fig. 2a, region D) was excised and used as probe to re-screen  $\lambda$ CH-01. The sea urchin H4 probe and the homologous H2A probe hybridized to  $\lambda$ CH-01 (Fig. 1c and f, respectively). No hybridization was observed with the sea urchin H3 probe (Fig. 1d). The H2A probe hybridized to two distinct regions (Fig. 1f), indicating the possibility that  $\lambda$ CH-01 contained two H2A genes.

### H3 and H2B genes identified in cDNA-positive regions of $\lambda$ CH-01 and $\lambda$ CH-02

As noted above, H3 and H2B heterologous probes were unsatisfactory for detailed analysis of the chicken histone clone  $\lambda$ CH-01. Fine hybridization mapping of the subclones pCH3.75H/E and pCH3.3E indicated two cDNA-positive regions, A and E (Fig. 2a), that did not hybridize with the available cross-species probes. Direct DNA sequence analysis identified these regions as H3 and H2B genes, respectively (Fig. 3).

The genomic clone  $\lambda$ CH-02 was originally identified as a histone gene clone on the basis of hybridization with a histone cDNA probe (ref. 1 and Fig. 2b). It did not hybridize with heterologous H2B or H4 probes (data not shown); however, the cDNA-positive regions F, G and H (Fig. 2b) did hybridize with H3 and H2B coding regions derived from  $\lambda$ CH-01. DNA sequence analysis of these regions showed that regions F and G of  $\lambda$ CH-02 contain adjacent H3 genes and that region H contains an H2B gene.

Thus, all cDNA-positive regions of  $\lambda$ CH-01 and  $\lambda$ CH-02 were shown to contain chicken histone genes. Portions of the nucleotide sequences which serve to identify each of these genes are shown in Fig. 3.

#### Location and orientation of histone genes

The precise location and orientation of all the core histone genes in  $\lambda CH$ -01 and  $\lambda CH$ -02 may be deduced from restriction enzyme mapping and DNA sequence analysis (Figs 2, 4). All core histone coding regions present in  $\lambda CH$ -01, except for one region in an H2A gene, have been fully sequenced (Fig. 4). In each case the nucleotide sequence predicts the expected amino

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pCH3.75H/E *(A) ATG GCG CGT ACG AAG CAG ACG GCG CAT AAG TCG
                 *(F) ATG GCG CGT ACG AAG CAG ACG GCG CGT AAG TCG
     pCH10E
                 *(G) ATG GCG CGT ACG AAG CAG ACG GCG CGT AAG TCG
     pCH10E
                          ala arg thr lys gln thr ala arg lys ser
                          alalarg thr lys gln thr ala arg tye ser 10
     CHICKEN H3
H2A
     pch3.75H/E *(B) ATG TCG GGG CGC GGG AAG CAG GGC GGG AAG GCG
                  *(D) ATG TCG GGG CGC GGA AAG CAG GGC GGG AAG GCG
     pCH3.3E
                          ser gly arg gly lye gln gly gly lye ala
                          ser gly arg gly lys gln gly gly lys ala 10
     CHICKEN H2A
H4
     pch3.75h/E *(C) ATG TCT GGC AGA GGC AAG GGC GGG AAG GGG CTC
                           ser gly arg gly lys gly gly lys gly leu
                           ser gly arg gly lys gly gly lys gly leu 10
     CALF H4
H2B
                  *(E) ATG CCC GAG CCG GCT AAG TCC GCG CCC GCC CCG
     pCH3.3E
                  *(H) ATG CCT GAG CCG GCC AAG TCC GCA CCC GCC CCC
     pCH4.7E
                           pro glu pro ala lys ser ala pro ala pro
                           pro<sup>1</sup> glu pro ala lys ser ala pro ala pro<sup>10</sup>
     CALF H2B
```

Fig. 3 Identification of histone genes in λCH-01 and λCH-02 by DNA sequence analysis. DNA sequences of all genes except the H2B gene in pCH4.7E were determined by the methods of Maxam and Gilbert<sup>33</sup>. Sequences from this gene were obtained by the methods of Sanger and Coulson<sup>34</sup>. The nucleotide sequences shown cover a region coding for the first 10 amino acids of each protein. The amino acid sequence for chicken H4 and H2B are not known. The genes for these proteins were assigned by comparing the amino acid sequences predicted in one reading frame with the respective calf amino acid sequences. The identifying DNA sequences are given in the order A to E and F to H shown as cDNA-positive regions of λCH-01 and λCH-02, respectively, in Fig. 2a and b. The extent to which nucleotide sequences have been determined in each coding region is shown in Fig. 4.

acid sequence for the respective histone protein. There are no intervening sequences or frameshift mutations and thus the genes located within  $\lambda CH$ -01 are not pseudogenes. The limited nucleotide sequence data for the H3 and H2B coding regions in  $\lambda CH$ -02 so far predict amino acid sequences expected for these two histones. The notable feature of  $\lambda CH$ -02 is the presence of adjacent, divergently transcribed H3 genes, separated by about 0.8 kbp of DNA. Although the H2A/H2B and H3/H4 gene pairs of yeast<sup>15</sup> and Drosophila<sup>12</sup> are divergently transcribed, this kind of organization for a histone gene pair coding for the same protein has not been described previously. The significance of the H3 gene pair disposition in the chicken system is unclear. By extrapolation from the recently described chorion gene system<sup>20</sup>, it is possible that the organization of divergently transcribed genes is significant for the expression of tissue-specific variants.

The overall arrangement of core histone genes within λCH-01 and  $\lambda$ CH-02 is shown in Fig. 5a and b, respectively. The two nserts are non-overlapping and although some clustering of genes occurs, there is no obvious tandem repeating unit. Within λCH-01, the H2A gene appears twice before a full complement of core histone genes is represented. The tightly linked H2A/H2B gene pair seen in λCH-01 is not present in λCH-02 and the adjacent H3 genes in ACH-02 are not related in an organizational sense to the H3 gene in  $\lambda$ CH-01. In other words, no two genes coding for the same histone are in equivalent environments. (These results refute our previous indication of a 15-kbp repeat for chicken histone genes using uncloned probes<sup>17</sup> and are consistent with preliminary analyses of other and are consistent with preliminary analyses of other chicken histone clones<sup>21</sup>.) The clones also contain long stretches of non-histone DNA. For example, in ACH-01, 9 kbp of DNA separate the H4 gene from the distal H2A gene (Fig. 5a) and an inter-gene distance of 4 kbp is seen in  $\lambda$ CH-02 (Fig. 5b).

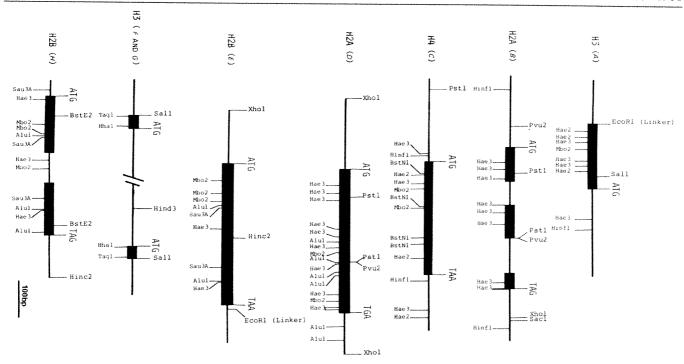


Fig. 4 The coding regions of genes in λCH-01 and λCH-02. Initiation and termination codon sites are shown for each gene where known. Restriction sites above the line can be used to orient the genes (see also Fig. 2). The blocked areas indicate coding regions for which the nucleotide sequence has been determined. Each gene fragment is identified A to H relative to the cDNA coding regions indicated in Fig. 2.

The relative disorder of histone genes in chicken may contribute to an understanding of histone gene evolution. The histones themselves represent highly conserved proteins with a defined function and it is unlikely that the mode of expression of histone genes would have changed dramatically through evolution. Preliminary data on a human histone gene clone (ref. 22 and unpublished data) suggest that marked histone gene disorder may also occur in other vertebrates. Although histone genes are frequently organized into ordered repeating units, there is little to suggest that this is a requirement for expression. The relative disorder of chicken histone genes also suggests that they are independently controlled. An explanation is therefore required to account for the evolution of histone genes from an ordered to a disordered disposition. It is possible that the concerted evolution of histone gene clusters in invertebrates is a function of chromosomal correction mechanisms acting on reiterated genes<sup>23-27</sup>, and that non-concerted evolution of chicken histone genes has arisen because the vertebrate gene copy

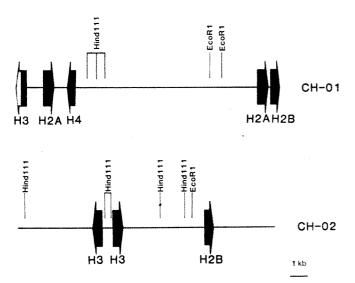


Fig. 5 Location and orientation of chicken histone genes. The precise location and orientation of genes in the two clones  $\lambda CH$ -01 and  $\lambda CH$ -02 relative to EcoRI and Hind3 restriction sites are indicated.

number is low<sup>7</sup> and correctional recombination events are less frequent. In this view, the gene organization in chickens is a remnant of the ordered arrangement of invertebrates, conserved in these species because they are more highly reiterated and not because of a functional requirement.

Clustering of histone genes in tandem arrays as first described for invertebrate systems remains the predominant histone gene organization in those species studied so far. Variations are seen in sea urchin orphons<sup>11</sup>, yeast<sup>15</sup> and Xenopus<sup>13,14</sup>. More recent data confirm that the major histone gene unit in *Xenopus* is a 14-kbp clustered array<sup>13,14</sup> and that the variants  $\lambda$ Xlh4 (ref. 13) and Xl-hi-1 (ref. 14) may be 'orphon like'. Unequal recombination events could result in the appearance of isolated genes or groups of genes, and these elements might be expected to show extensive population polymorphism as is indeed the case for sea urchin histone gene orphons<sup>11</sup> and the minor histone gene components of *Xenopus*<sup>14</sup>. It is unlikely that the chicken histone genes analysed here represent orphons. Southern blot analysis (ref. 21 and our unpublished data) is not consistent with the existence of a major repeating histone gene cluster in the chicken genome.

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### Sequence homologies near the C-termini of the variable surface glycoproteins of Trypanosoma brucei

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African trypanosomes evade their mammalian host's immune system by the sequential expression of alternative surface glycoproteins. Comparison of the cDNA sequences of five antigenically distinct surface proteins and the C-terminal amino acid sequences of four additional ones demonstrates that these nine glycoproteins can be classified into two subsets based on C-terminal amino acid homologies that extend 75-105 amino acids into the protein.

UNICELLULAR parasites of the genus Trypanosoma cause extensive disease in both humans and livestock1. In Africa the disease is especially widespread among domestic livestock and thus severely limits the consumption of meat and milk products by over 200 million people living south of the Sahara Desert2. The parasite resides in the bloodstream of its mammalian host and appears to evade the host's immune response by sequentially expressing a series of different surface glycoproteins, the variable surface glycoproteins (VSGs)<sup>3,4</sup>. The largest documented number of immunologically distinct VSGs that can arise from a single trypanosome clone is 101, reported for Trypanosoma equiperdum<sup>5</sup>; the maximum potential number is unknown

VSGs isolated from Trypanosoma brucei are best characterized. The VSGs constitute at least 95% of the surface protein of this organism and as much as 10% of the total cell proteins<sup>6</sup>. T. brucei VSGs have molecular weights between 55,000 and 65,000 and most of those studied have widely different amino acid compositions, dissimilar peptide maps, different isoelectric points and variable carbohydrate content<sup>7-11</sup>. In addition, the N-terminal sequences of 15-30 amino acids reported for five different VSGs are very different and have no obvious relationship<sup>3,9</sup>. Nevertheless the different VSGs do have a similar biological function. They comprise a cloud or shell that shields other membrane proteins from the host's immune system. As such, each collection of unique VSGs must be able to assume a uniform and closely ordered array on the surface of the organism.

Here we compare five different VSG cDNA sequences and, together with the C-terminal amino acid sequences of four others, demonstrate that the VSGs characterized so far can be grouped into two major subsets based on amino acid homologies near the C-terminus.

#### Origin of the VSG cDNAs

The ILTAT series of T. brucei clones are derived from LUMP 227<sup>12</sup>, which originated from a trypanosome stock isolated in 1964 from a cow in Kenya<sup>13</sup>. They are serially related as follows.

LUMP 227 
$$\rightarrow$$
  $\rightarrow$  ILTAT 1.1  $\rightarrow$  ILTAT 1.2

ILTAT 1.4

In each case the ILTAT clone was isolated from the first

teppanosome relapse population in an experimental animal infected with the previous clone: for example, ILTATs 1.3 and 14 were each isolated from a laboratory mouse infected with ILTAT 1.2. The construction, identification and initial characterization of the four ILTAT VSG cDNAs are described elsewhere 14-1

The partial sequence of VSG 117 cDNA, shown in Fig. 1, was recently reported by Boothroyd et al. 18. Trypanosome clone 117 is derived from another T. brucei stock isolated in 1960 from a sheep in Uganda19.

#### VSG nucleotide coding sequence homologies

Sucleotide sequences of VSG cDNAs of ILTAT 1.2, ILTAT 1.3 and 117 are compared in Fig. 1; the sequences of ILTAT 1.1 and 1.4 cDNAs are compared in Fig. 2. No direct amino acid sequence information is available on the ILTAT VSGs. However, the amino acid sequences of each can be deduced from the nucleotide sequences as each contains only one large translation reading frame uninterrupted by termination codons. The largest cDNA, ILTAT 1.3, extends 1,515 nucleotides from the 5' end of the cDNA to the (DNA) termination codon TAA, followed by 87 nucleotides (including three additional termination codons in phase) before the poly(A) region is reached. The corresponding protein of 505 amino acids has a molecular weight (MW) of 54,886, which is within the range of native VSGs, if adjusted for the addition of carbohydrate moieties and the potential proteolytic processing of an N-terminal signal sequence<sup>20</sup> and the hydrophobic portion of the C-terminus<sup>18</sup> Unfortunately there is no definitive way to determine from the nucleotide sequence alone whether one of the two methionine odons near the start of the ILTAT 1.3 coding sequence is the ranslation initiation codon. The 16 hydrophobic amino acids hat immediately follow the second of these methionine residues ossibly represents an N-terminal signal peptide although there s no experimental evidence for this conclusion. The other DNAs are smaller and do not contain the entire VSG coding

Comparison of the nucleotide sequences in Figs 1 and 2 eveals striking homologies among VSG coding sequences preiously thought to be unrelated based on nucleic acid hybridizaion studies<sup>21</sup>, N-terminal amino acid analyses<sup>3</sup>, and antibody ross-reactivity<sup>6,12</sup>. In Fig. 1, the sequences of ILTAT 1.3 and 17 cDNAs were initially compared by aligning the termination odons of their open translation reading frames. The ILTAT 1.2

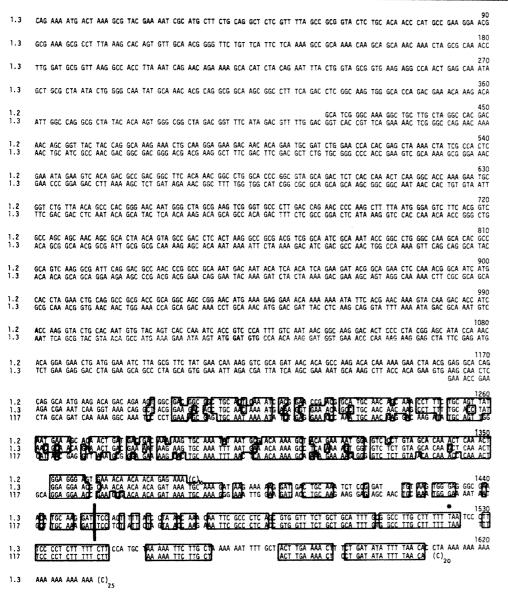
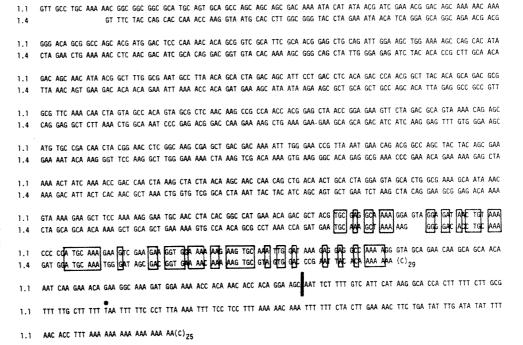
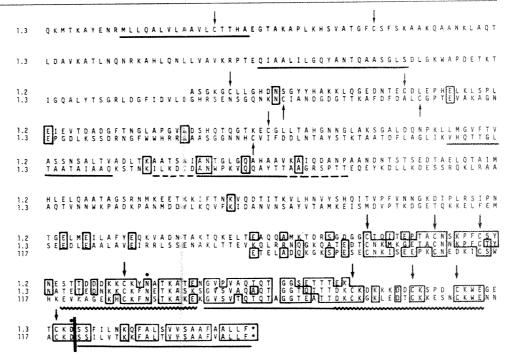


Fig. 1 Nucleotide sequences of ILTAT 1.2 and 1.3 cDNAs compared with the VSG 117 cDNA sequence as reported by Boothroyd et al. 18. Sequence determinations were carried out using the methods of Sanger<sup>30</sup> and Maxam and Gil-bert<sup>31</sup>. Most areas were verified two to four times by sequence overlaps. (Sequencing strategy and duplicate autoradiograms of sequencing gels are available on request.) The three sequences are aligned to maximize nucleotide homologies; this necessitated treating two different triplets in the coding region of VSG 117 as insertions relative to ILTATs 1.2 and 1.3 and four different locations in the 3' untranslated region of VSG 117 as deletions relative to ILTAT 1.3. The boxed regions indicate nucleotide homologies within the last 330 nucleotides. No significant homologies were detected before this region. The asterisk shows the termination codons. The region between the vertical bar and the asterisk codes for a 23-amino acid hydrophobic tail (see Fig. 3).

Fig. 2 Nucleotide sequences of ILTAT 1.1 and ILTAT 1.4 cDNA aligned to maximize the homology that occurs near the 3' end of ILTAT 1.4 cDNA. This necessitated treating one triplet in ILTAT 1.1 as an insertion relative to ILTAT 1.4. Sequences were determinined by the method of Maxam and Gilbert<sup>31</sup> Boxed regions indicate homologies within the last 100 nucleotides of ILTAT 1.4 cDNA. No significant homologies were detected before this region. The region between the vertical bar and the asterisk codes for a 17-amino acid hydrophobic tail (see Fig. 5).



3 Amino acid sequences ILTAT 1.2, ILTAT 1.3 and VSG 117 as deduced from the nucleotide sequences shown in Fig. 1. The C-termini of ILTAT 1.3 and VSG 117 are indicated by an asterisk. Boxes show amino acid homologies. Horizontal and wavy lines underneath the sequences indicate hydrophobic or uncharged hydrophilic regions, polar and respectively. Broken lines indicate regions that are uncharged polar or hydrophilic in one sequence but not in the other. Arrows indicate the cysteine residues. Dots show two glycosylation sites in VSG 117<sup>26</sup> The large vertical bar indicates the proposed cleavage site of a 23-amino acid hydrophobic tail from the nascent VSG.



sequence was then aligned to maximize homology with the other two sequences. The homology among the three cDNAs starts  $\sim$ 330 nucleotides before the termination codon and extends through the 3' untranslated region to the poly(A) terminus at the 3' end. Based on this alignment, the ILTAT 1.2 cDNA sequence ceases  $\sim$ 140 nucleotides before the termination codon.

The cDNA sequences of VSG 117 and ILTAT 1.3 both contain the 3' untranslated sequence between the termination codon and the poly(A) region (although the VSG 117 sequence seems to stop two nucleotides before the poly(A) region). These two 3' untranslated regions are virtually identical except for four deletions of 1, 3, 7 and 13 nucleotides in VSG 117 (or four insertions in ILTAT 1.3). The remaining 59 nucleotides in both sequences differ at only two positions.

The ILTAT 1.1 sequence (Fig. 2) also includes the 3'-terminal poly(A) region of the mRNA. The distance from the termination codon to the poly(A) in this case is 84 nucleotides. Alignment of all three untranslated regions to maximize homology (not shown) reveals that 42 positions are identical in the three VSG mRNAs, mostly near the poly(A) end. The significance of these observations is unclear but it does seem that portions of the 3' untranslated region do not tolerate much mutational drift. Similar sequence conservation has also been observed in the 3' noncoding region of different haemagglutinin genes of influenza virus RNA<sup>22</sup>. In contrast, the different mammalian  $\beta$ -globin genes have a high degree of sequence divergence in this region<sup>23</sup>.

Aside from the 3' untranslated region and the last three codons of the coding region, ILTAT 1.1 cDNA does not possess distinctive homology with the cDNAs of ILTAT 1.2, ILTAT 1.3 and VSG 117 shown in Fig. 1. However it does have similarities to ILTAT 1.4 cDNA. As aligned in Fig. 2, 61 of the last 100 nucleotides of ILTAT 1.4 are the same as the corresponding nucleotides in ILTAT 1.1. On this basis, the ILTAT 1.4 cDNA stops ~123 nucleotides before the termination codon. Therefore if this homology extends to the termination codon of ILTAT 1.4 coding sequence, it comprises ~225 nucleotides (or 75 codons).

### Amino acid sequences of VSGs are conserved near the C-terminus

Figure 3 compares the deduced amino acid sequences of ILTATs 1.2, 1.3 and VSG 117. These comparisons indicate that amino acid homologies near the C-terminus parallel and extend beyond the nucleotide sequence homologies noted in Fig. 1.

Furthermore, many of the amino acid changes near the C-termini are conservative in that an amino acid of similar polarity has been substituted. Generally, the C-terminal 105 amino acids of all three VSGs are highly conserved, the next 15 positions are less conserved, and a comparison of ILTAT 1.2 and ILTAT 1.3 through the next 390 amino acids towards the N-terminus reveals no significant amino acid homology (although there may be some conservation of domains as discussed below).

Figure 4a illustrates the common features of the three sequences. Within the highly-conserved C-terminal 105 amino abids are eight cysteine residues whose relative positions in the three VSGs are constant. This feature is very similar to that of the influenza virus surface glycoprotein, haemagglutinin, which also undergoes antigenic variation and contains eight invariant cysteines in the last 100 amino acid residues<sup>24</sup>. Air and Hall<sup>25</sup> have examined 35 different haemagglutinins of 17 different influenza subtypes and found that the cysteine residues are invariant throughout the entire protein. This suggests that disulphide linkages have an integral role in maintaining a general three-dimensional conformation of variants of both trypanosome VSGs and influenza haemagglutinins.

The last 23 codons of the ILTAT 1.3 and VSG 117 coding sequences specify a very hydrophobic region; seven of these amino acids are different in the two sequences but only one

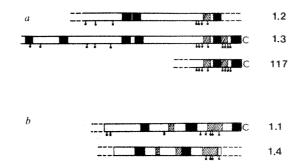


Fig. 4 Summary of the conserved features of a, ILTATs 1.2 and 1.3, and VSG 117 and b, ILTATs 1.1 and 1.4. C indicates the C-terminus; solid boxes are hydrophobic or uncharged polar regions; cross-hatching indicates highly hydrophilic regions; \$\ddot\$, cysteine residues. Broken horizontal lines represent regions for which no sequence information is available.

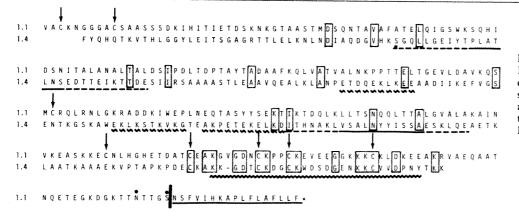


Fig. 5 Amino acid sequences of ILTAT 1.1 and ILTAT 1.4 as deduced from the nucleotide sequences shown in Fig. 2. Symbols are the same as for Fig. 3. The C-terminal hydrophobic tail in ILTAT 1.1 is 17 amino acids long.

change involves a charge difference at neutral pH. Boothroyd et al. 18 and Holder and Cross 26 reported that this 23-amino acid hydrophobic sequence is not present in the mature VSG based on C-terminal amino acid analysis of VSG 117. Furthermore, they found that the C-terminal residue is glycosylated. (The cDNA sequence of both ILTAT 1.3 and VSG 117 indicates that this terminal residue is aspartic acid, which implies an unusual glycosylation linkage.) The deduced ILTAT 1.3 amino acid sequence is identical to the VSG 117 sequence at this proposed cleavage and glycosylation site; this suggests that both proteins undergo similar processing events. The hydrophobic C-terminal sequence is preceded by a 25-26-amino acid region that is extremely hydrophilic and contains four of the invariant cysteine residues. This hydrophilic region is preceded by a conserved uncharged polar region of 17-18 residues that, in turn, is preceded by another hydrophilic region containing the other four invariant cysteine residues. The amino acid sequences then begin to diverge and very little homology is detected beyond 120 amino acids from the C-termini. Nevertheless, as Fig. 4a shows, ILTAT 1.2 and ILTAT 1.3 are similar in this divergent region in that three more sulphydryl residues and two additional uncharged polar regions occur in the same relative locations.

Figure 5 compares the deduced amino acid sequences of ILTATs 1.1 and 1.4 and Fig. 4b illustrates their common features. Although clearly similar near the C-termini, these two VSGs are less homologous than the other three. The positions of the four cysteine residues closest to the C-termini are conserved within a very hydrophilic region, similar to the other VSGs. But ILTAT 1.1 has additional cysteines further upstream that are not present in ILTAT 1.4. In addition, these two VSGs do not seem to have within their homology a distinctly conserved uncharged polar region flanked by two hydrophilic regions containing cysteines, as do the others.

ILTAT 1.1 has a very hydrophobic C-terminus as do ILTAT 1.3 and VSG 117. As inferred from other data<sup>26</sup> on VSG C-terminal amino acids, the last 17 amino acids are not present in the mature ILTAT 1.1. A comparison of this 17-amino acid hydrophobic 'tails' with the 23-amino acid hydrophobic 'tails' of ILTAT 1.2 and VSG 117 shows two similar features in addition to the hydrophobicity. All three terminate with Leu-Leu-Phe and possess a lysine seven residues removed from the proposed cleavage and glycosylation site. The significance, if any, of these similarities must remain speculative.

In summary, the C-terminal homologies of all five VSGs shown in Figs 3-5 preserve the occurrence of a C-terminal hydrophobic domain and the positions of either four or eight cysteine residues within very hydrophilic regions, suggesting that these regions are required for formation of a general three-dimensional structure. No sequence homologies larger than two amino acids occur within the N-terminal portions of the glycoproteins.

#### VSG subsets based on C-terminal homology

The above discussion suggests that VSGs can be classified into subsets based on C-terminal homology, much the same as the isotype classification of immunoglobulins (IgM, IgG, etc.) even

though the mechanisms of VSG and immunoglobulin gene expression are different 15,27,28. This possibility is substantiated by a recent report 26 of the amino acid sequences of tryptic glycopeptides at the C-termini of VSG 117 and four other VSGs. These sequences and the corresponding ILTAT sequences are listed in Fig. 6. Clearly, the nine immunologically distinct VSGs for which C-terminal information is available fall into two groups based on these sequences. Holder and Cross 26 obtained sequences of internal glycosylated tryptic fragments and these sequences generally support the same grouping into two subsets. Similarly, genomic DNA hybridization experiments using the Southern procedure also support the conclusion that there are sequence subsets of VSGs 15.28. In low-stringency hybridization conditions, C-terminal fragments of VSG cDNA hybridize to many regions in the genomic DNA; under high stringency, or using a cDNA containing N-terminal sequences, only one or a few coding regions are detected.

Finally, the genomic DNA hybridization experiments have indicated that at least two different types of DNA rearrangements occur around most, if not all, VSG coding sequences in the trypanosome genome <sup>14,18,29</sup> and may be involved in the mechanism of VSG expression. In some cases, an 'expression-linked extra copy' (ELEC) of a VSG gene occurs in cells expressing that gene<sup>18,29</sup>; in other cases, complex DNA rearrangements beyond the 3' untranslated region of the gene seem to occur, rather than an ELEC<sup>14,18,29</sup>. These two different types of DNA rearrangement do not correlate with the two different sequence homology subsets. For example, VSG 117, ILTAT 1.2 and ILTAT 1.3 belong to the same VSG sequence subset but an ELEC is associated with VSG 117 expression while 3'-distal rearrangements occur next to the ILTAT 1.2 and 1.3 coding sequences.

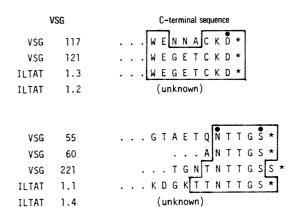


Fig. 6 Comparison of the C-terminal amino acid sequences of nine different VGSs (taken from Figs 3 and 5 and ref. 26). The exact C-terminal sequences of ILTATs 1.2 and 1.4 are unknown but they are placed in their respective groups on the basis of sequence homologies further into the protein, as shown in Figs 3 and 5. \*, C-termini; ●, glycosylation sites described in ref. 26.

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### Is a bi-stable beam responsible for the complex radio structure of 3C133?

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It is generally assumed that the extended lobes of radio emission found in many extragalactic sources are powered by oppositely directed supply beams of relativistic particles emanating from the central galactic nucleus<sup>1,2</sup>. The complexity and asymmetry of the structure in many sources has, however, led to speculation of a 'flip-flop' beam mechanism<sup>3,4</sup> where energy is supplied alternately to each radio lobe. Recent high resolution observations of the radio structure of 3C133 reveal a complex structure in which rotation of the source axis may have uncovered evidence for such a bi-stable nature of the energy supply. There are, however, several difficulties in such an interpretation.

The source was observed at 1,666 MHz for 13 h in August 1980, using five telescopes of the multi-telescope radio linked interferometer (MTRLI) at Jodrell Bank<sup>5</sup>. The data have been analysed by CLEAN and closure phase techniques<sup>6</sup>. The results are presented as a contour map in Fig. 1 and the main parameters of the components are summarized in Table 1. A restoring beam of 0.4 arcs, rather than the full resolution of 0.25 arc s, has been used to display the extended regions of low surface brightness.

There are four main regions of radio emission. An unresolved central component (C) is coincident with a 21st magnitude galaxy of redshift 0.28 (ref. 7). There is only a single component (D) to the west, but there are two to the east. One of these (B) is very diffuse, and the other (A) is compact and is connected to the nucleus by a narrow jetlike feature. Emission from the heads of all four components has been detected in observations of lower dynamic range with the Cambridge 5-km telescope at 15.4 GHz (ref. 8).

The lack of reflection symmetry in the structure of 3C133 is difficult to explain in terms of twin supply beams which form outer lobes at 'working surfaces' where the beams impinge on the external medium<sup>1,2</sup>. Any such explanation assumes that there have been at least two outbursts from the central object. In addition, a contrived situation is required in which motion of the galaxy through the external medium9 is coupled with either relativistic doppler effects<sup>10</sup>, beam instabilities<sup>11</sup> or an anisotropic distribution of electron pitch angles12 to account for the misalignments and number of observed components.

If the outer components were formed at different times, their morphologies might be used as indicators of their relative ages. In such a basis, the compact component A would be the oungest (and this is supported by the still evident jet), the more extended component D would be of intermediate age, and the diffuse component B, which resembles an outer lobe of well evolved source, would be the oldest. There may thus have been three outbursts of activity in 3C133, and as none of the components has a companion of similar age on the opposite side of the nucleus, these outbursts may have been fundamentally one-sided rather than symmetric in nature.

If this is so, the misalignments of the outer components could be explained by a rotation of the source axis similar to that observed in other sources 13,14. The brightness distributions across the heads of the outer components are all markedly asymmetric and have their peaks offset in a manner suggestive of clockwise rotation. This implied sense of rotation is substantiated by the observation that the jet is directed to the northern edge of component A.

While it is possible to envisage a one-sided beam which has swung through an angle  $>360^{\circ}$  and has had long quiescent periods between the outbursts, the overall configuration of the

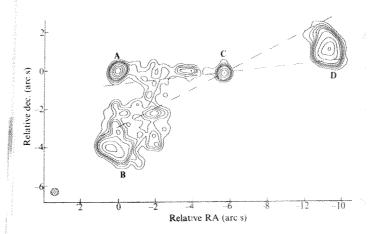


Fig. 1 The MTRLI map of 3C133 at 1,666 MHz. North is to the top and east is to the left. The restoring beam of 0.4 arcs is shown as a shaded circle to the bottom left. The contours are 2, 4, 6, 8, 10, 15, 30, 50, and 75% of the peak brightness of 225 mJy per beam. The broken lines drawn as a guide for the eye are in position angles 94° and 111°.

Table 1 Parameters of components of 3C133

Component	Separation from nucleus (kpc)*	Range of position angles subtended from nucleus (deg)†	Mean component size (kpc)*†	Flux density at 1.7 GHz (mJy)
C Jet to	0		<3	205
component	0-30	86-88	*******	45
A D B	30 31 38	85-93 94-111(+180) 114-130	7 13 19	450 1,020 (1,510)‡

<sup>\*</sup> Calculated from the source redshift for an Einstein-de Sitter cosmology with  $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ .

source and the run of position angles subtended by the outer components (Table 1) can be more readily explained by a one-sided beam which has rotated through ~35° and has undergone two 180° flips in direction.

The possibility of a bi-stable beam mechanism has been suggested by recent dynamical modelling<sup>15</sup> of an initially spherically symmetric ejection of plasma from the neighbourhood of a supermassive black hole which is embedded in a flattened confining gas cloud. Calculations show that a slight displacement of the black hole from the gas cloud centre will form a one-sided jet along the direction of easiest escape. If the black hole oscillates through or rotates around the gas cloud, there could be a periodic reversal in the direction of the jet.

The observational data allow limits to be set on the time scales of precession and beam reversals for 3C133. From Fig. 1, the position angles of components A and B (measured from C) differ by ~35°, but any bending of the jet is ≤2°. Therefore, if the rotation of the jet is uniform in the plane of the sky, the rotation period  $(T_R)$  must be  $>(360^\circ/2^\circ)\times$  (jet travel time from components C to A), that is,  $>2\times10^7$  yr. The extent of the transverse elongations of components B and D imply that the interval between flips is  $\sim 0.05 T_R$ , that is,  $> 10^6$  yr which is

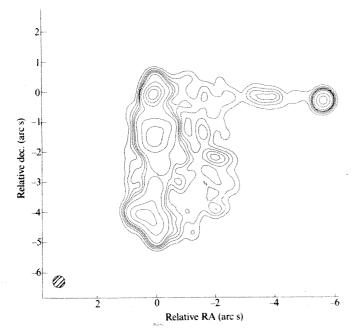


Fig. 2 MTRLI map of 3C133 after the D component has been reflected through the central component. The contours and the restoring beam are the same as for Fig. 1.

consistent with time periods of rotation and z oscillation of galaxies as would be implied by the Wiita and Siah model. In Fig. 2, the major effects of the beam reversals have been artificially removed by reflecting the D component of Fig. 1 through the central component to produce a near-continuous ridge of emission. If the small gaps between the components are due to the evacuation of new channels by the beam, the time scale for the reversal is  $\sim 0.02 T_R$  and the component re-evacuation speed is  $\leq 0.25c$ .

At first sight, the radio structure of 3C133 apparently provides the strongest observational evidence for the existence of a bi-stable or 'flip-flop' beam mechanism. There are, however, two main difficulties with such an hypothesis.

First, if the ordering of relative ages is correct, then the measured flux densities given in Table 1 indicate that the oldest components are the most luminous. If the greater size of the older components is attributable to adiabatic expansion<sup>16</sup>, then their past luminosities would have been up to several hundred times greater than at present, implying that the energy supply from the galactic nucleus has decreased dramatically with time over the three outbursts.

Second, the derived beam reversal time scale implies that the age of component B is  $> 2 \times 10^6$  yr. There is therefore a problem in explaining the detection of emission from all the outer components at 15.4 GHz, as the synchrotron lifetimes of the electrons radiating predominantly at this frequency are  $\sim 3 \times$ 10<sup>5</sup> yr if the magnetic fields and electrons are in equipartition. This difficulty may be overcome if the rotation is non-uniform, as might be expected if the beams are collimated in the vicinity of a Kerr black hole whose spin axis was initially misaligned with that of the surrounding galaxy<sup>17</sup>. If the axes initially differed by ~40°, but have now swung into near alignment because of the interaction between the angular momenta of the black hole and of the galactic material, then the rotation would originally have been much more rapid, removing the apparent discrepancy between the implied component ages and the synchrotron lifetimes. The similarity of the angles subtended by the components would then imply that the time between beam reversals would have lengthened considerably.

If both the beam luminosity and the length of time that the components are powered have varied, then it seems fortuitous that the outer components all lie at similar distances from the nucleus. Perhaps a simpler solution to the difficulties might be that particle reacceleration occurs in the outer components to produce a delayed brightening of the outer heads after the beam is switched off. It is, however, difficult to envisage such a physical process, and it seems ironic that the need for in situ particle acceleration in outer lobes of radio sources was the reason why beams providing a continuous supply of energy were originally postulated.

Despite some problems, a bi-stable beam mechanism seems to offer the most natural explanation for the unusual and complex structure of 3C133. Note, however, that the evidence for such a mechanism is only conspicuous because of the rotation of the source axis. To what extent such switching occurs in normal co-linear sources is unclear, but such a mechanism could explain the complex sub-structure seen in many sources<sup>18</sup>, and the one-sided emission of the more compact and possibly younger D2 objects<sup>19</sup>.

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<sup>†</sup> Component sizes defined by 20% contours of their peak brightnesses

<sup>‡</sup> The flux density of this component is almost certainly underestimated due to the limited spatial frequency coverage of the obser-

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### Unidentified features in the spectrum of Triton

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Water frost has been identified on the surfaces of the satellites of Jupiter<sup>1</sup>, Saturn<sup>2</sup> and Uranus<sup>3,4</sup>; methane frost is dominant on . However, although the IR spectrum of Triton, the largest satellite of Neptune, is similar to that of methane or methane frost<sup>8</sup>, this identification is not consistent with all the available data<sup>9,10</sup>. We have now used the Multiple Mirror Telescope (MMT) to obtain an improved IR spectrum of Triton. Our observations show features that are not in detailed agreement with the identification of methane, although the general spectral behaviour that led to this identification is confirmed. A satisfactory identification of the surface and/or atmospheric composition on Triton does not yet seem possible.

Observations were made with the circularly variable filter in the MMT IR photometer. The wavelength calibration and spectral resolution of 1% were determined by laboratory measurements of gaseous discharge lamps and were confirmed by measurements of P $\beta$  and B $\gamma$  from the planetary nebula NGC7027. All observations were made through a 9 arcs aperture and with reference beam spacing of 20 arc s. Care was taken to ensure that neither the measurement beam nor the reference beams were contaminated by light from Neptune. Observations were carried out on 24.4 April, 13.4 June and 16.4 June 1981 (UT), in all cases very close to maximum elongation of Triton from Neptune. Atmospheric corrections were determined by measuring the solar type star 58 Oph at equal elevation to Triton.

Figure 1a shows the spectrum of Triton, Fig. 1b the same data smoothed to 4% spectral resolution. The error bars are  $\pm 1$  s.d. and have been estimated from the reproducibility of repeated spectral scans, on the assumption that the noise level (dominated by detector noise) was the same for all wavelengths. The longest total integration time was devoted to the 1.94-2.12-µm region of the spectrum; hence, the signal-to-noise ratio is highest there.

The new data are in satisfactory agreement with previous narrow-band photometry of Triton<sup>9</sup>. A previous fully sampled but very noisy spectrum of Triton<sup>9</sup> agrees in broad outline with the new data but seemed to show high resolution structure that is only partially confirmed by the higher quality data presented here

The data show the broad absorption near 2.35 µm which led to the previous identification of methane<sup>8</sup>. There is also a narrower, but resolved, absorption at 2.10 µm, and possibly an unresolved absorption at 2.00 µm, an absorption or absorption edge near 1.94 µm and a broad but shallow absorption at 1.65 µm. These latter three features are at a 2-3 s.d. level of significance and need to be confirmed at higher signal-to-noise ratio. In addition, the very strong and broad absorption from 3 to 4 µm (ref. 11) is confirmed by a new measurement with the Infrared Telescope Facility, which shows the average reflectivity between 3.0 and 3.8  $\mu$ m to be  $0.48 \pm 0.05$  times the reflectivity at 2.2 µm. In the visible, the spectrum of Triton is featureless except for a general decrease in reflectivity towards the  $UV^{10,12}$ 

The features in the spectrum of Triton could arise directly from the surface material or from gases in a tenuous atmosphere, or from some combination. The lower limit of 0.19 for the albedo12 suggests that the surface may be at least partially frost-covered. Therefore, we have considered the reflectance spectra<sup>13</sup> of four frosts that are among the most likely candidates for this environment (Fig. 1): H<sub>2</sub>O, CH<sub>4</sub>, NH<sub>3</sub> and H<sub>2</sub>S. These spectra have been smoothed to correspond to a resolution of ~1%. None of the frosts provides a satisfactory match to the spectrum of Triton. CH4 and H2S frosts are probably also too reflective from 3 to 4 µm to be consistent with the observations of Triton. We have failed to find a satisfactory match to the spectrum of any other frost.

At the temperature appropriate for the surface of Triton, only a few molecules have sufficiently high vapour pressure to form an atmosphere. Of these, the only two which are likely to be present on the basis of normal solar abundances and which are spectrally active in the 1.6-2.4 μm region are CH<sub>4</sub> and CO. Either of these gases can account for the broad absorption near  $2.35 \ \mu m$ ; however, CH<sub>4</sub> would then produce an absorption of nearly equal strength from 1.65 to 1.8  $\mu m,$  and CO would not be

absorbing between 3 and 4  $\mu$ m. Recent studies<sup>10</sup> have placed an upper limit of  $1 \times 10^{-3}$ amagat on the atmospheric CH4 abundance on Triton. The absence of any absorption centred at 1.7 µm in our spectrum places an independent limit of  $1.5 \times 10^{-3}$  amagat on the atmospheric CH<sub>4</sub> abundance. The absorption feature near 2.35 μm is two to three times stronger than would correspond to an atmosphere of CH4 at these limits. It therefore seems unlikely that CH<sub>4</sub> as either gas or solid is the dominant spectrally active constituent on Triton.

A combination of surface and atmospheric absorptions might resolve some of these discrepancies-for example, many hydrated minerals and frosts are very dark between 3 and 4  $\mu m$ , so that a CO (and possibly methane) atmosphere over a rocky or frosty surface might fit the data. However, the absorption feature at 2.10 µm remains an anomaly. Very few of the

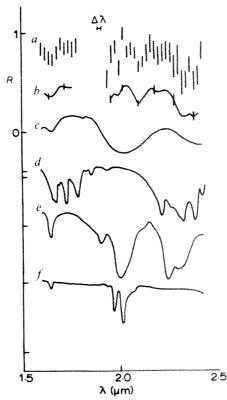


Fig. 1 Reflectivity of Triton and selected frosts. a, Arbitrarily normalized reflectivity of Triton at the original spectral resolution of 1.0%. Error bars are ±1 s.d. b, Data of a smoothed to 4% resolution. The zero point for this and the subsequent curves are indicated by tick marks on the vertical axis. c, Reflectivity of H<sub>2</sub>O frost. d, Reflectivity of CH4 frost. e, Reflectivity of NH3 frost. f, Reflectivity of H2S frost.

molecules likely to be abundant on Triton produce features at this wavelength, and the few which do (such as N<sub>2</sub>O) do not match the remainder of the spectrum satisfactorily.

The surface compositions of small bodies in the outer Solar System have generally followed a simple pattern: a single material from a small list of those likely to be abundant dominates their IR reflection spectra. Triton seems to violate this pattern. Further improvements in the observations and in the laboratory spectra of candidate materials will be required to analyse the surface of Triton.

The Multiple Mirror Telescope Observatory used for the present research is a joint facility of the University of Arizona and the Smithsonian Institute. The photometry at 3.5 µm was taken with the Infrared Telescope Facility, which is operated by the University of Hawaii under contract from NASA. Assistance with the observations was provided by B. Kindred and B. Light. We acknowledge helpful discussions with D. Cruikshank, U. Fink and G. Sill. This work was supported by NASA.

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### Morphology of iodine-doped polyacetylene

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Although the synthesis of polyacetylene powders was first carried out many years ago, Ito et al.1 have only recently obtained films which can be doped with various chemical species, such as I2, SbF5 or Na, K, giving p or n type semiconductors. These and other properties, such as its optical bandgap<sup>2</sup> ( $E_g \approx 1.5 \text{ eV}$ ) have made polyacetylene an interesting material for study3mechanical stretching ability, for example, leads to partially oriented fibrils and an electrical conductivity anisotropy  $\gamma_{\parallel}/\gamma_{\perp} \simeq 10$  has been measured with  $\gamma_{\parallel}$  reaching  $3 \times 10^3 \, \Omega^{-1} \, \mathrm{cm}^{-1}$  when highly doped with AsF<sub>5</sub>; roughly 12 orders of magnitude higher than the value measured on undoped cis (CH)<sub>x</sub>. Polyacetylene is available in two isomeric configurations (cis and trans, Fig. 1). More than 98% cis isomer is obtained if the synthesis is performed at low temperatures (-78 °C) but it is not thermodynamically stable and becomes trans on increasing the temperature, the total transformation occurring when T > 100 °C. Any doping process induces a partial cis/trans isomerization<sup>8</sup>. The exact conduction mechanism is poorly known: the localization and nature of the doping species have been widely discussed. Here we investigate, using scanning electron microscopy, the behaviour of the morphology of (CH), films when the dopant concentration varies. Starting from the fibrillar structure we have observed the formation of aggregates and a uniform melt of various globular features at high doping levels. Our results yield information on how the electrical conductivity takes place in these non-uniform materials.

Acetylene was polymerized, using Shirakawa's technique1, on the surfaces of a glass reactor. The essential experimental conditions are: solvent, toluene; Al/Ti ratio of 4; catalyst concentration, 0.3 Ti mol 1<sup>-1</sup>; ageing of catalyst, 1 h; temperature, -78 °C; acetylene pressure, 1 atm. The 100-μm thick films obtained were carefully washed in toluene. The cis-rich (CH)<sub>x</sub> samples were doped with iodine vapour in equilibrium with its liquid phase at 25 °C, samples were then pumped for 2 days to remove the interfibrillar non-fixed dopant molecules. The dopant concentrations were evaluated from the weight increase. At low doping levels, the concentration y, defined by (CHI<sub>v</sub>)<sub>x</sub>, is a mean value over the whole sample, taking into account the fact that the macroscopic diffusion coefficient of iodine in  $(CH)_x$  films is  $\sim 10^{-8}$  cm<sup>2</sup> s<sup>-1</sup>. All our observations were made in the middle of the section of the films to avoid any boundary effect, and the samples were fractured under liquid nitrogen to avoid stresses.

Gold coating was performed by sputtering, the thickness of the obtained film being in the range 50-100 Å. Scanning electron micrographs were performed using a JEOL JSM 35 instrument working with an electron beam energy of 20 keV, and a current <1 nA to avoid any radiation damage.

On the first micrograph (Fig. 2a) we observe the fibrillar structure of the undoped cis-rich (CH)<sub>x</sub>, where a few globular features appear. For  $y \approx 10^{-2}$  (Fig. 2b) we note white areas as well as the formation of large aggregates. With y = 0.03 (Fig. 2c) we observe: (1) an extension of the large aggregates; (2) a regression of the fibrillar structure, which seems to be masked: (3) the appearance of many small white points. We also observe that the true volume occupied by the material is enhanced along Fig. 2a to c. For y = 0.14 (Fig. 2d), all the platelets and fibrils are recovered with the white points whose size has increased. With y = 0.3 (Fig. 2e) the observed background is very homogeneous and spattered with large white points.

It has been shown elsewhere that the fibrillar structure is modified when the dopant concentration increases, so we performed another experiment to find out whether the fibrils were masked or destroyed. A (CH)<sub>x</sub> film was iodine doped up to y = 0.30 and then the dopant was extracted by plunging the doped film in toluene with granular silver to form AgI. After a week, the dopant concentration in the film had fallen to y = 0.1: Fig. 2f shows clearly that the fibrillar structure remains, although their length is reduced and their diameter enhanced; it seems that the y = 0.1 dopant concentration only applies inside

Using a CF<sub>3</sub>SO<sub>3</sub>H dopant we do not observe white points but a considerable increase in size of the fibrils at high dopant levels leading to the formation of islands.

We have investigated the dopant diffusion mechanism during the doping process of (CH), films and shown that the usual diffusion Fick law's cannot explain the measured dopant profiles through the film—an additional process related to a chemical interaction between dopant molecules and (CH)<sub>x</sub> fibrils must be involved which must include two kinds of dopant inhomogeneity: (1) A macroscopic inhomogeneous distribution of doping molecules, as presented above, which disappear on highly doped samples. (2) An inhomogeneity inside the fibrils, which persists for a long time, due to the very low diffusion of

Fig. 1 Cis (a) and trans (b) isomeric configurations of polyacetylene.

dopant molecules in the crystalline parts of the (CH)x films, but leads to a rapid production of the observed platelets and to an increase of the diameters of the remaining fibres.

Finally note that the y values for Fig. 2b-e are an approximative evaluation, the uncertainty is due to the dopant inhomo-

geneity through the sample discussed above.

Recent experiments10 and I2 and SbF5 doping trans (CH)x seems to produce a more homogeneous doped material but these SEM observations were performed at the film surfaces and not on the cross-section. We have also observed that the surface composition and morphology are uniform at all doping rates9, and nearly the same on either side of the film.

To correlate our observations with the electronic transport properties of doped (CH)x, we have plotted on Fig. 3 the electrical conductivity and the corresponding activation energy against the dopant content3 which exhibits a semiconductor to metal transition near 1-3%, depending on the dopant species. At lower dopant levels Rice<sup>11</sup> proposed a theoretical model for the electrical conductivity based on the assumption of a very inhomogeneous distribution of the doping species inside the fibrils. In all cases the conducting process was attributed to an extrinsic semiconducting process where an acceptor A or a

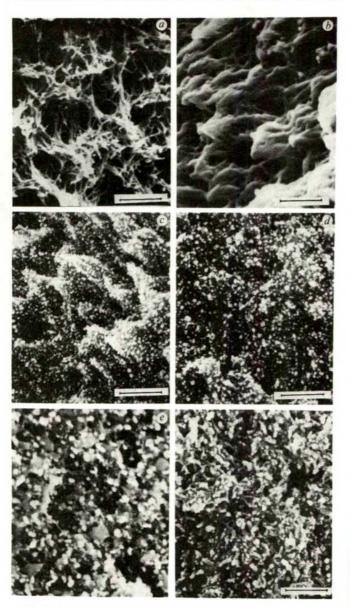
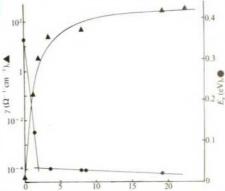


Fig. 2 Scanning electron micrographs of cross-sections of (CH)<sub>x</sub> films (150 µm thick) sputter-coated with 80 Å of gold. Scale bar for all micrographs, 1 µm. a, Undoped cis (CH)x; b, iodine doped  $(CHI_y)_x$  with y = 0.01; c, y = 0.03; d, y = 0.1; e, y = 0.3. f, Sample of e after a partial desorption of the dopant, residual y = 0.1.



Typical behaviour of electrical conductivity and deduced Fig. 3 activation energy of doped (CH)x (from ref. 3).

donor A+ lying near a chain bring, by thermal activation, a carrier which can be delocalized along the chain.

Looking at our present data, taken at various doping levels, some previous assumptions should be revised.

- (1) At very low dopant concentrations  $y < 10^{-2}$  (Fig. 2b) the presence of fibrils coated with doping species seems likely. However, a chemical reaction between the bulk material and the doping species leads to a rapid production of the observed platelets.
- (2) For higher concentrations an important modification of the polyacetylene morphology takes place: fibrils seem modified. In addition, the increasing size and number of white globules with increasing dopant concentration (Fig. 2e) suggest a percolation process to explain the conductivity mechanism (Fig. 3). The slight conductivity increase observed above y =0.03 would be due to the variation of the size and conductivity of the elementary clusters. However, these results are obtained when cis (CH), film is doped at room temperature (25 °C) using the dopant vapour phase in equilibrium with its solid one. We have also verified that similar results are obtained by doping with a pentane solution of iodine if the dopant concentration is high enough.

Consequently we can analyse these observations by way of an inhomogeneous doping process: vapour iodine condense on the fibrils and rapidly react with the polymer to give very highly doped regions. The percolation process could be a consequence of this dopant concentration inhomogeneity in the sample.

These assumptions are also compatible with the effect of dopant concentration on the thermoelectric power and far IR absorption. Note that such a percolation model, implying conductivity between metallic particles embedded in a dielectric continuum, has also been proposed by Mortensen et al.

In conclusion our first experiments, using scanning micrograph analysis, on the doping effect on the polyacetylene morphology indicate that, at very low  $(y \le 10^{-2})$  dopant concentration, the dopant may produce the same effect as that of an impurity on an inorganic classical semiconductor of increasing the carrier number; at higher concentration, that is, around the semiconductor to metal transition region and above, the fibrillar structure seems modified.

Elsewhere the fibrillar structure evolves leading to short fibrils, the diameter of which is largely increased. Consequently the morphology of the doped material strongly depends on the polymerization and doping conditions.

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## Fusion of pyrope at high pressures and rapid crystal growth from the pyrope melt

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Pyrope Mg<sub>3</sub>Al<sub>2</sub>Si<sub>3</sub>O<sub>12</sub> is the magnesium end member of the pyralspite garnet series, which is an important constituent of the upper mantle. Melting experiments at high pressure are necessary to clarify the chemical heterogeneity in the deep mantle and the origin of various magmas. Some authors have suggested the possibility of the pressure-induced structural change of silicate melts and discussed its importance on the magma generation and the differentiation in the mantle<sup>1,2</sup>. We report here melting experiments on pyrope at pressures up to 10 GPa as a first stage in extending the pressure range of model systems for the upper mantle and examining the possibility of the pressure-induced structural change of the melt at high pressure. The correlation between the melting curve and the change of the quenched phases from the melt is the first evidence of a structural change of the pyrope melt at high pressure.

Pure pyrope synthesized at 6 GPa and 1,250 °C was used as the starting material. This powder was directly packed in a graphite capsule which is used as a heater. An MA8-type high-pressure apparatus<sup>3</sup> was used. Pressure calibration was

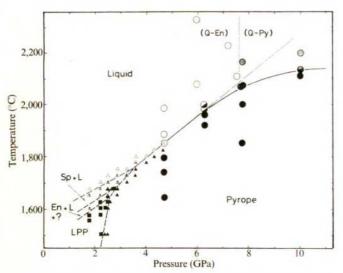


Fig. 1 Melting curve of pyrope up to 10 GPa. The data below 4.7 GPa are from ref. 5 and above this pressure are from the present work. LPP, low pressure phases (mixture of sapphirine, alumonous enstatite, and sillimanite); En+?+L, aluminous enstatite, undetermined phase (sillimanite or sapphirine?) and liquid; Sp+L, spinel and liquid; ●, pyrope recrystallized without melting; O, quench crystal of aluminous enstatite formed from the melt by quenching (Q-En) (glass is also observed below 6.5 GPa); O, quench crystal of pyrope formed from the melt by quenching (Q-Py). The pressure at the change of the quenched phase is shown as a dotted line. The light dashed curve is the extrapolated melting curve fitted to data below 7.0 GPa with the Kraut-Kennedy equation. The curve is expressed as  $T(K) = 1,708 (1 + 9.5\Delta V/V)$ , where  $\Delta V/V$  is calculated using the Birch-Managhan equation of state with the available low-temperature bulk modulus and its pressure derivative9. The discrepancy becomes very large above 7.5 GPa.

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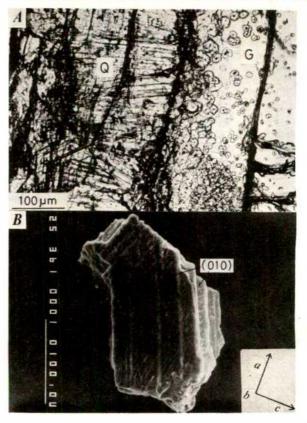
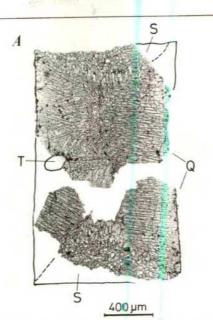


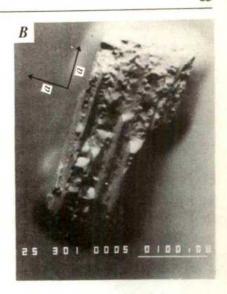
Fig. 2 A, Photomicrograph of the run product quenched from 5 GPa and 1,850 °C. The glassy part (G) and the lamellae of quench aluminous enstatite (Q) are clear. B, Scanning electron image of quench aluminous enstatite. The lamellar texture and the (010) plane are clear. The elongated direction of the lamellae does not coincide with the c-axis, and the angle between the elongated direction and the c-axis is  $69.3 \pm 0.5^{\circ}$  in this lamellar crystal. The streaks on the (010) plane are also observed in this photograph. Scale bar,  $100 \, \mu \text{m}$ .

made at high temperature using the phase boundaries of olivinespinel transition in Fe<sub>2</sub>SiO<sub>4</sub> and coesite-stishovite transition determined by a high-pressure X-ray diffraction method4. Temperature was measured by a W-W26% Re thermocouple. The quenching method was used to determine the melting curve. The furnace assembly was dried in an oven at about 150 °C for a few hours immediately before each run to avoid the effect of absorbed water on the melting temperature. Pressure was applied to the sample first, and then the temperature was brought to a desired value. Heating was limited to a few minutes to avoid the contamination by the furnace material and the thermocouple to the sample. The sample was quenched isobarically by shutting off the electric power supply. Thin sections of the run products were prepared for microscopic observation and EPMA analysis. X-ray diffraction was also used to identify the run products. Melting was recognized by microscopic observation of the texture of the run products.

Figure 1 shows the melting curve of pyrope up to 10 GPa. The results below 4.7 GPa were obtained by Boyd and England<sup>5</sup>, whereas the data above 4.8 GPa come from the present investigation. As the temperature gradient of the furnace is large; the difference of the temperatures between the central part and the end part of the furnace amounted to ~100 °C (ref. 7) only the run products around the thermocouple were selected and examined to determine the melting curve shown in Fig. 1. The present results combined with those of Boyd and England indicate that pyrope melts congruently from 3.6 to 10 GPa. The slope of the melting curve is very large, about 79° GPa<sup>-1</sup> at pressures below 7 GPa; above this pressure there is a significant decrease. The possibility that the bend of the melting curve originates from an ambiguity of the present pressure scale can be

Fig. 3 A, Photomicrograph of the run product quenched from 10 GPa and 2,200 °C. The quenched pyrope with lamellar texture (Q) is observed clearly. The recrystallized pyrope without melting (S) observed in the end part of the furnace cell is due to the temperature gradient of the furnace. T is the point of the thermocouple. B, The backscattered electron image (TOPO) of quenched pyrope. Pyrope quench crystal elongates to [100] direction. Scale bar, 100 μm.





ruled out, because we made the pressure calibration at high temperature using the phase boundaries determined by X-ray diffraction on the basis of NaCl pressure scale<sup>4</sup> and such bend of the melting curve above 7.0 GPa was not observed in the case of forsterite<sup>6</sup> and fayalite<sup>3</sup> for which the same apparatus and pressure scale were used.

The pyrope melt is quenched to a glass and quench crystal with a lamellar texture below 6.5 GPa. Above this pressure, the run products formed from the melt were aggregates of quench crystal, and the glass was not observed. The quench crystal was aluminous enstatite at pressure below 7.5 GPa. Above 7.5 Gpa quench crystals of pyrope were formed in place of aluminous enstatite.

Table 1 X-ray diffraction and chemical data of aluminous enstatite quench crystal

			question or y		
h	k	1	$d_{\mathrm{obs}}\left(\bar{\mathrm{A}}\right)$	$d_{\mathrm{cal}}\left(\mathring{\mathbf{A}}\right)$	$I_{\mathrm{obs}}$
0	2	0	4.319	4.322	67
1	2	1	3.266	3.267	17
4 2 3 6 5	2 2 2 2 2 1	0 1	3.124	3.130 3.119	50
2	2	1	2.910	2.911	36
6	1	0	2.856	2.856	43
5	1	1	2.811	2.813	21
		1	2.680	2.680	14
7	2	1)	2.000	2.495	
4 1 2 5 4 3 7 5 5 7 1 4	2 3 0 2 3	1 }	2.495	2.494	100
. 5	2	1	2.451	2.450	14
4	3	0	2.431	2.433	7
3	0	2	2.383	2.384	14
3		1	2.325	2.326	9
7	3	1	2.241	2.240	10
5		1	2.071	2.070	10
5	1	2	2.050	2.050	10
7	2	2	2.045	2.044	9
1	3 1 2 4	1	1.9843	1.9831	14
4 2	4	0	1.9506	1.9512 1.9485	14
2		1	1.9359	1.9359	10
10	1	0	1.7757	1.7765	9
2	5	0	1.6985	1.6984	10
6	3 1 5 5 3 3	0	1.5019	1.5011	11
1	3	3	1.4777	1.4778	17
10	3	1	1.4727	1.4727	17
0	6	0	1.4402	1.4408	86

Cell constants: a,  $18.152 \pm 0.006 \, \text{Å}$ ; b,  $8.645 \pm 0.002 \, \text{Å}$ ; c,  $5.188 \pm 0.002 \, \text{Å}$ ; V,  $814.06 \pm 0.48 \, \text{Å}^3$ . Space group, Pbca. SiO<sub>2</sub>,  $45.13 \, \text{wt\%}$ ; Al<sub>2</sub>O<sub>3</sub>,  $24.83 \, \text{wt\%}$ ; MgO,  $29.80 \, \text{wt\%}$ ; total,  $99.76 \, \text{wt\%}$ . Numbers of ions on the basis of six oxygens:

Si 1.517 Al 0.483 2.000 Al 0.501 Mg 1.492 1.992

Figure 2A shows the coexistence of glass and quench crystals formed by quenching from 1,850 °C at 5 GPa. Both the glass and the quench crystal were analysed by EPMA and found to have a pyrope composition. There is no difference in the chemistry of the quench crystal and the glass. The X-ray diffraction pattern of the quench crystal can be indexed as an orthorhombic pyroxene with a space group Pbca. According to the EPMA and X-ray diffraction analysis (Table 1), the quench crystal is an aluminous enstatite with a pyrope composition. To our knowledge, this is the first report of a pyroxene with pyrope stoichiometry. The scanning electron image (SEI) of the quenched aluminous enstatite is shown in Fig. 2B where the lamellar texture and (010) plane are clear. The elongated direction of lamellae does not coincide with that of tetrahedral chains along the c-axis of the pyroxene structure, and the angle between the elongated direction and the c-axis varies from 60° to 70° among the different sheaves of lamellae.

The quenched pyrope formed at pressures above 7.5 GPa has a lamellar texture and can be distinguished from the pyrope crystal recrystallized without melting, which is granular in texture. Figure 3A shows the coexistence of a quenched pyrope and a recrystallized pyrope. The coexistence of these two phases is one of disequilibrium and is caused by the temperature gradient in the furnace. The difference in the textures is clear. X-ray precession photographs indicate that the lamellae elongate parallel to [100] direction and the planes parallel to [100], which are assigned to {011} planes, are also observed. The backscattered electron (TOPO) image of the quenched pyrope is shown in Fig. 3B. The quench crystal from the pyrope melt changes from aluminous enstatite to pyrope around 7.5 GPa in accordance with the bend of the melting curve.

The bend of the melting curve can be explained by a volume decrease of the melt due to a pressure-induced structural change. The structural change of the melt by a shifting of Al ions from fourfold to sixfold coordination has been suggested by Waff<sup>2</sup>. Recent Raman spectrometry of the jadeite glass synthesized at pressures up to 4 GPa indicated that there was no evidence of a coordination change of Al ions7. However, this type of the structural change in the melt can be expected to occur at even higher pressure. Al ions occupy both tetrahedral and octahedral sites in aluminous enstatite, while all Al ions occupy the octahedral sites in pyrope. As has been reported, some pyroxenes transform to the garnet structure at high pressure8. Then the change of the quench crystal from pyroxene to garnet in accordance with the bend of the melting curve may suggest such a pressure-induced coordination change of ions in the pyrope melt at high pressure.

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### Hydrazines and carbohydrazides produced from oxidized carbon in Earth's primitive environment

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Our ideas of the possible composition of the atmosphere of the primitive Earth have broadened markedly since Oparin's and Urey's<sup>2</sup> postulates of a highly reducing atmosphere containing hydrogen, ammonia, hydrocarbons and water. Since then, various geochemical models of the primordial atmosphere have been proposed<sup>3-6</sup>. Of importance is whether abiological organic compounds can be formed from the interactions of energy sources with nitrogen, oxidized carbon and water. Experiments involving electrical discharge<sup>5,7</sup> and bombardment with beams of He ions<sup>6</sup> of various mixtures of CO, CO<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub> and H<sub>2</sub>O suggested that formic acid was the major organic product. We now report experiments using quenched spark discharges through molecular nitrogen on aqueous suspensions of CaCO<sub>3</sub> and other reactants to simulate the atmosphere/hydrosphere interface. Hydrazine and carbohydrazide are recovered in significant but low yields. Their reactions in primitive aquatic environments could have supplied a pathway for chemical evolution and the origin of life on a primitive Earth in which carbon in the fully oxidized states was available for the primary synthesis of organic matter.

Our experiments were designed (1) to supply aqueous inorganic carbon in the form of calcium carbonate to effect pH control near pH 7.5-8.0 as well as to provide dissolved CO<sub>2</sub> and dissolved CO<sub>3</sub><sup>2-</sup> anion in constant small amounts determined by the low solubility product of CaCO<sub>3</sub>, and (2) to supply Fe<sup>0</sup> to act as a scavenger for oxidizing agents. We believe that such a system corresponding to a primordial atmosphere/hydrosphere interface is suitable for assessing potential pathways for organic synthesis where carbon is in the +4 oxidation state.

Five-litre flasks equipped with a central nichrome or gold wire electrode and a high-vacuum valve were filled with 50 ml doubly distilled water, 100 mg (1.78 mmol) 400-mesh reduced iron and 100 mg (1 mmol) CaCO<sub>3</sub>. The gas phase was evacuated, purged with nitrogen and filled with 750 mm nitrogen (223.2 mmol). For some experiments, 0.5 mCi NaH<sup>14</sup>CO (specific activity 50 Ci mol<sup>-1</sup>) was added using a gas-tight syringe through an attached septum. An Electro-Technics highfrequency generator was connected to the central electrode and discharged on the water surface through a capacitatively coupled external metal ground plate. After 10-18 h, the cloudy white suspension of CaCO<sub>3</sub> became clear and the pH dropped from 8.1 to 7.0. At this time, gas chromatographic analysis of the headspace (5 ml samples at ~750 mm) showed no detectable H<sub>2</sub> or O<sub>2</sub>, traces of CO<sub>2</sub> and minute amounts of CH<sub>4</sub> in an excess of unreacted nitrogen.

Chemical analysis of both the water and particulate phases

was negative for nitriles (by pyrohydrolysis and benzidine acetate-copper(II) acetate staining) and for amino acids (by ninhydrin staining). Nitriles present in nanomolar amounts could have been detected by this method. TLC on silica gel plates revealed a single dominant organic component which was detected by iodine vapour, or by rhodamine-B staining, or as a dark spot on silica gel layers containing a 254-nm UV fluorescent dye. The product had the same  $R_{\rm f}$  values as standard carbohydrazide in four solvent systems: (1) butanol/formic acid/water, 17:1:2; (2) CHCl<sub>3</sub>/methanol/8.4 M NH<sub>4</sub>OH, 2:2:1; (3) CHCl<sub>3</sub>/methanol/12 M NH<sub>4</sub>OH, 85:15:1; and (4) CHCl<sub>3</sub>/methanol/8.4 M NH<sub>4</sub>OH, 1:4:1. Standard carbohydrazide and a related standard, semicarbazide, were obtained from the Aldrich Chemical Co.

When 0.5 mCi NaH14CO3 was included in several such quenched spark-discharge reaction mixtures, 30% of the organic 14C-labelled products migrated with R<sub>f</sub> values identical to carbohydrazide in the above TLC solvent systems, while the balance remained at the origins. (The organic carbon products remaining at the origins have not been firmly identified, although when subjected to 6 M HCl hydrolysis at 120 °C, ninhydrin-positive and UV chromatophores were released.) In one typical experiment, 1,200 d.p.m. total organic carbon per 40-μl sample  $(1.5 \times 10^6 \, d.p.m.$  per  $50.5 \, ml$  total reaction volume) were recovered after addition of 6 M HCl at 25 °C to decompose unreacted carbonate. Of this total,  $0.42 \times 10^6$  d.p.m. (28%) behaved identically to carbohydrazide on the chromatograph. If we assume both labelled NaHCO3 and unlabelled CaCO, to be equally reactive, the carbohydrazide yield was 0.38 µmol, and the total organic carbon yield 1.38 µmol. This latter value indicates conversion of 0.14% of the initial carbonate to organic carbon.

Carbohydrazides are used as intermediates in various organic synthetic reactions<sup>8,10,11</sup> and undergo many reactions similar to hydrazine. Hydrazine is implicated as an intermediate in the reactions leading to carbohydrazide formation, because carbohydrazide (III) is a major carbon-containing product and its synthesis results from reactions of hydrazine (I) with carbon dioxide via carbazic acid (II) as an intermediate 9,10:

$$\begin{array}{lll} H_2NNH_2 + CO_2 \rightarrow H_2NNHCO_2H \rightarrow H_2NNHCONHNH_2 + HOH \\ & \text{(III)} \end{array}$$

Tests for hydrazine in the water phase of these reactions were positive, as determined by TLC of its yellow p-dimethylaminobenzaldehyde azine derivative and quantitatively using the ASTM standard test D-1385-18 for hydrazine in water, and confirmed by combined gas chromatographic/mass spectrometric analysis. Table 1 presents the data for the synthesis of hydrazine in various reaction conditions. Assuming linearity, the maximal rate of synthesis was 0.83 µmol hydrazine h<sup>-1</sup>; the rate decreases after  $\sim 10$  h, coincidentally with the apparent loss of solid CaCO<sub>3</sub>.

Table 1 also summarizes data on the presence of carbohydrazide, the final pH and other observations for various combinations of reactants in quenched spark discharge experiments. Note that hydrazine is always present with carbohydrazide. Carbonate is required and the pH must be maintained near or above 7.0 during most of the hydrazine/carbohydrazide synthesis. Reduced iron is not required to scavenge oxidants, but dinitrogen appears to fulfil this role, as manifest by the relatively large amounts of nitrite and nitrate seen in all experiments. The data so far do not indicate whether solid carbonate or soluble carbonate anions are involved when CaCO<sub>3</sub> is used. If carbon is present as CO<sub>2</sub>, no hydrazine nor carbohydrazide is formed. Neither Ca2+ nor Na+ anions will lead to the synthesis of hydrazine or carbohydrazide in the absence of carbonate. When air was substituted for nitrogen, hydrazine and carbohydrazide yields were similar to the anoxic experiments. In all cases, carbohydrazide is only found when hydrazine is synthesized, supporting our suggestion that its synthesis proceeds via carbazic acid, as proposed by others9,16

These experiments show that, in anoxic prebiotic or contemporary atmospheric conditions, in the absence of molecular

Table 1 Products obtained by quenched spark discharge on various reactants

Reactants	Products (yields are total material recovered per flask after 10 h sparking)	(Initial) final pH
A		
750 mm N <sub>2</sub> ; 1 mmol CaCO <sub>3</sub> ; 1.78 mmol Fe; 50 ml H <sub>2</sub> O	5.86 μmol hydrazine; 0.5 μmol carbohydrazide; 870 μmol NO <sub>2</sub> ; 1,130 μmol NO <sub>3</sub>	(8.1) 7.3
750 mm N <sub>2</sub> ; 1 mmol CaCO <sub>3</sub> ; 50 ml H <sub>2</sub> O	8.28 μmol hydrazine; 0.5 μmol carbohydrazide; 316 μmol NO <sub>2</sub> ; 74 μmol NO <sub>3</sub>	(8.1) 6.6
750 mm N <sub>2</sub> ; 1 mmol NaCO <sub>3</sub> ; 1.78 mmol Fe; 50 ml H <sub>2</sub> O	6.84 μmol hydrazine; 0.5 μmol carbohydrazide; 265 μmol NO <sub>2</sub> ; 1,675 μmol NO <sub>3</sub>	(8.2)
750 mm air; 1 mmol CaCO <sub>3</sub> ; 50 ml H <sub>2</sub> O	5.07 μmol hydrazine; 0.5 μmol carbohydrazide; 640 μmol NO <sub>2</sub> ; 1,080 μmol NO <sub>3</sub>	(8.1) 7.1
В		
750 mm N <sub>2</sub> ; 1.78 mmol Fe; 50 ml H <sub>2</sub> O	<0.02 µmol hydrazine; carbohydrazide, not detected; >500 µmol NO <sub>2</sub> ; >1,000 µmol NO <sub>3</sub>	(6.0) 4.2
$750~mm~N_2;30~mm~CO_2;\\50~ml~H_2O$	<0.01 μmol hydrazine; carbohydrazide, not detected; >500 μmol NO <sub>2</sub> ; >1,000 μmol NO <sub>3</sub>	(5.2) 1.1
$750~mm~N_2; 20~mm~CO_2; \\ 1.78~mmol~Fe$	<0.01 µmol hydrazine; carbohydrazide; not detected; >500 µmol NO <sub>2</sub> ; >1,000 µmol NO <sub>3</sub>	(5.0) 1.1
750 nm N <sub>2</sub> ; 1 mmol CaCl <sub>2</sub> ; 50 ml H <sub>2</sub> O	<0.01 µmol hydrazine; carbohydrazide, not detected; NO <sub>2</sub> /NO <sub>3</sub> not measured	(5.0) 1.4
750 mm N <sub>2</sub> ; 1 mmol NaCl; 50 ml H <sub>2</sub> O	$<\!0.01~\mu mol$ hydrazine; carbohydrazide, not detected; $NO_2/NO_3$ not measured	(5.0) 1.4
Control 750 mm N <sub>2</sub> ; 1 mmol CaCO <sub>3</sub> ; 50 ml H <sub>2</sub> O; No spark	$<\!0.01~\mu mol$ hydrazine; carbohydrazide, not detected; $NO_2/NO_3$ not detected	(8.0)

A: those reaction conditions which lead to the synthesis of hydrazine and carbohydrazide. B, those reaction conditions which lead to the oxidation of molecular nitrogen. Nitrate and nitrite were measured according to ref. 15.

hydrogen: (1) molecular nitrogen can be reduced to hydrazine; (2) oxidized carbon is reduced to organic carbon products of which one-third is carbohydrazide; (3) molecular nitrogen serves as the primary oxidant scavenger, leading to the formation of nitrite and nitrate.

The significance of these simulated processes in the routes to organic matter from oxidized carbon in a mildly reducing prebiotic Earth model remains to be assessed. Nonetheless, the yields reported here, though lower than in similar systems containing methane<sup>13</sup>, are much higher than achieved earlier in systems containing fully oxidized carbon<sup>5,7</sup>. This suggests that organic synthesis in prebiotic surface environments consonant with previous models<sup>3-6</sup> merits further investigation. More productive processes may yet be found; further studies of similar heterogenous systems are underway

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### Temperature and precipitation record in southern Chile extended to ~43,000 yr ago

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Southern Chile (41-56°S), in the belt of westerly winds, receives mostly heavy precipitation, reaching 8,500 mm yr near 50 °S and decreasing northwards and southwards from this latitude to ≤1,500 mm; mean January (summer) temperatures near sea level along the latitudinal gradient are 8-16 °C (refs 1, 2). Dense rain forest covers much of the region to 48 °S; south to Cape Horn, magellanic moorland prevails and rain forest becomes limited. Modern pollen measured in surface samples reflects the distribution of plant species in the vegetation. We have now applied regression equations relating present-day pollen to temperature and precipitation to fossil pollen data at Taiquemó to assess climatic conditions during the Quaternary. The results extend our previous record of the past 16,000 yr at Alerce<sup>3</sup> beyond the lateglacial to  $\sim$ 43,000 yr BP. For the 27,000-yr interval, they show mean January temperatures of 10-12 °C and mean annual precipitation centred around 1,000 mm except during the time span of ~31,000-43,000 yr BP when amounts increased to 4,000 mm. In general fluctuations correspond to the isotopic climatic reconstruction in Antarctica4 and to changes inferred from pollen data at comparable latitudes in Tasmania<sup>5-8</sup> and New Zealand<sup>9,10</sup>.

We derived temperature and precipitation data from the pollen contained in sediments in a lake basin at Taiquemó (42°10'30" S, 73°35'45" W)<sup>11</sup>. The site is located on northeastern Isla Chiloé at an elevation of 170 m, 105 km south-west of Alerce where we reported quantification of southern Chile climatic change to 16,000 yr ago<sup>3</sup>. The basin is situated at the outer edge of an end moraine that is partially cutover and occupied by remnants of Valdivian rain forest. The moraine seems to date from the middle part of the Llanquihue (Wisconsin) Glaciation, the last glacial age to affect Isla Chiloé. A 760-cm core from the basin is  $^{14}$ C dated to  $42,700 \pm 1,200$ (QL-1011) and to 42,400±1,000 (QL-1012) yr BP at the respective 700-cm and 760-cm levels; these and six additional ages establish the chronology of the core sediments.

Data for the sediments in the core (Table 1) show peat beds in the upper 200 cm and below 560 cm interbedded by gyttja. The upper bed began forming at 14,120 yr BP and the lower bed formed from ~43,000 until before 31,200 yr BP. Loss on ignition measurements indicate that percentages of organic matter in the core are lowest during the lacustrine phase between 200 and 560 cm; the sedimentation rate over the 43,000-vr interval was lowest during the latter part of this phase from 20,100 to

14010 1 Sedimentary data for the Taiguemá ope	Table 1	Sedimentary data for the Taiquemó core
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Depth (cm) 0-160	Sedimentary material	% Average weight loss on ignition	Age interval	Sedimentation (yr cm <sup>-1</sup> )	Average pollen density (grains cm <sup>-3</sup> )	Average pollen influx (grains cm <sup>-2</sup> yr <sup>-1</sup>
160-200 200-260 260-300 300-400 400-490 490-700 700-760	Peat Peat Gyttja Gyttja Gyttja Gyttja Gyttja-peat Peat-sand	80 84 27 9 14 18 31 63	0-12,000 12,000-14,120 14,120-20,100 20,100-21,900 21,900-26,600 26,600-31,200 31,200-42,700 42,700-42,400	75 53 100 45 47 51 55 55*	$36 \times 10^{3}$ $87 \times 10^{3}$ $39 \times 10^{3}$ $20 \times 10^{3}$ $22 \times 10^{3}$ $59 \times 10^{3}$ $211 \times 10^{3}$ $423 \times 10^{3}$	500 1,600 400 400 500 1,200 3,800

<sup>\*</sup>For this interval, the same rate as between 490 and 700 cm is assumed.

14,120 yr BP. Pollen influx is relatively low; averages are higher for the peat than for the gyttja, with highest values in the basal peat.

The temperature and precipitation equations were produced by stepwise regression, using normalized per cent averages of 20 pollen taxa as predictors. Although the taxa are moderately intercorrelated (maximum r = 0.69), orthogonalization of the variables failed, even after rotation, to produce a biologically convincing solution. A total of 59 composite sites (160 surface samples) was used for estimating temperature and 57 for precipitation. Addition of 33 composite sites from the rain forest of Tierra del Fuego<sup>12</sup> enlarged the data set on which Alerce estimates were based. Application of the new equations to the Alerce data gave results in agreement with trends reported previously. The new equations seem, however, to be much more sensitive to observed variations in the older pollen record at Taiquemó. The temperature equation has seven terms that explain 82% of the variance; the standard error of estimate is 0.96 °C (12% of the range of surface temperatures). The precipitation equation has nine terms accounting for 86% of the variance; the standard error is 415 mm (11% of the range of surface values). Plots of observed versus predicted temperature and precipitation indicate that the equations are reasonably structured within the limitations of the surface data set. Pollen spectra occasionally occur which seem to correspond with vegetation from warmer and wetter sites outside our surface data range. The equations predict excessively warm and wet conditions at these levels. Expansion of the surface data base would probably remedy this but samples are not available. Similarly, there seems to be some distortion of the record at the lower temperatures. Despite these limitations, we believe that trends are correctly described.

When applied to the fossil pollen stratigraphy at Taiquemó, the equations produce curves (Fig. 1) that can be fitted to the trends of temperature and precipitation between ~12,000 and 16,000 yr BP at Alerce. Only this portion of overlap in the two sets of data is shown to simplify the fit; the younger portions of the Taiquemó curves, represented by only 16 stratigraphical levels, lack a chronology and the detail shown by the 73 levels at Alerce. Accordingly, we have omitted data younger than 12,000 yr BP at Taiquemó in favour of the entire Alerce record.

Results indicate a mean January temperature of  $\sim 10$  °C between 14,120 and 31,200 yr BP. This followed a decrease from 12 °C around 43,000 yr ago. Minimum temperatures over the time span are  $\sim 4$  °C lower than present-day ones at Taiquemó. Precipitation is <1,000 mm from  $\sim 14,000$  until 21,900–26,600 yr BP; values are greatest before 31,200 and register a distinct rise to >4,000 mm around 43,000 yr BP. The range over which precipitation changes is >3,000 mm and is >1,500 above and below the modern mean of 2,600 mm. The long intervals with limited change seem unusual but are consistent with the minimal frequency variations shown in the pollen profiles at Taiquemó<sup>11</sup>.

Interstadial conditions at Taiquemó were clearly wetter and warmer around 43,000 yr BP. Precipitation has fluctuated less

but noticeably since then until ~14,000 yr BP, while changes in temperature are only  $\leq$ 1 °C. The minor increase in precipitation with no perceptible increase in temperature between 26,600 and 31,200 yr BP represents an apparent climatic event. The interval is also recognized in the pollen stratigraphy at Río Teguaco, 13.5 km directly south of Taiquemó, where it is  $^{14}$ C dated to between 25,500 ± 500 (QL-1017) and 30,000 ± 300 (QL-1019) yr BP. Drier and colder climate existed at Taiquemó during a stade that lasted until at least 14,000 yr ago. These climatic changes seem consistent with what is broadly known of glacier fluctuations in the region during the Llanquihue Glaciation  $^{11,13,14}$ .

Overall relationships can be drawn between the behaviour of the curves at Taiquemó and climatic conditions recognized from other data sources in the middle and higher latitudes of the Southern Hemisphere. The estimated 32,000-yr oxygen isotope

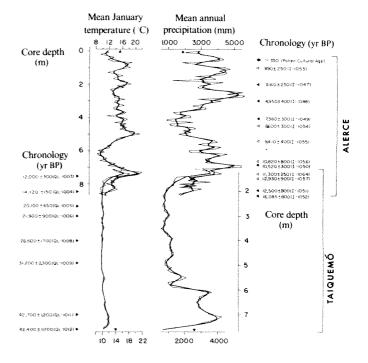


Fig. 1 Mean January temperature and mean annual precipitation at Alerce (upper scales) and Taiquemó (lower scales) for the past ~43,000 yr. Bold lines represent three-point moving averages of the data. Triangles on the temperature and precipitation scales indicate the modern means; <sup>14</sup>C ages are also indicated by triangles (closed ones relate to the cores whereas open ones for Alerce are from sites nearby that are stratigraphically correlated). Alerce and Taiquemó scales are offset slightly to align the curves.

stratigraphy for the Dome C ice core from east Antarctica4 indicates trends that are approximately parallel despite the degree of uncertainty in the Dome C chronology, which is taken from 14C ages of a marine core in the Indian Ocean. Major isotopic shifts indicate slightly ameliorated conditions before 25,000 yr BP, cold with decreased snow accumulation until ~16,000 yr BP (the time of the maximum advance of the West Antarctic Ice Sheet between 21,200 and 17,000 14C yr BP)15 and finally a warming trend that became stabilized close to 10,000 yr BP with minimal changes thereafter. Because of the uncertainty of the Dome C chronology, it is not possible to interpret certain changes in the stratigraphical record. This difficulty also applies to comparison of marine data with the Chilean data; however, estimated sea surface temperature profiles, derived from radiolarian assemblages in conjunction with oxygen isotope stratigraphy in marine sediments of the subAntarctic, do show the same trends as those at Taiquemó over what is interpreted to be the same time interval<sup>16</sup>

Pollen data from Tasmania<sup>5-8</sup> and New Zealand<sup>9,10</sup> in the zone of westerly winds (41-43 °S) are probably more relevant to the Taiquemó sequence of interstadial and stadial climates. At Blakes Opening in south-central Tasmania, a wetter and to some extent warmer interstade, when temperate rain forest developed, is <sup>14</sup>C dated earlier than 41,150 <sup>+1,45</sup><sub>-1,25</sub> -53,400 yr BP; later, wet sclerophyll forest succeeded in cooler and less humid conditions. The same climatic changes are apparent at Pulbeena and Mowbray Swamps in north-west Tasmania where grassy eucalyptus woodland or scrub in a cool, moist climate before 35,000 yr BP was followed by grassland associated with cold and lower humidity through the lateglacial. In northwestern South Island, New Zealand, the cold, relatively dry climate indicated by grassland continued until ~12,000 yr ago, except for an interstade characterized by shrubland with some spread of southern beech from ~26,000 until before 31,000 yr BP. Within the time limits set by the 14C chronology, these successive climatic events correspond remarkably with those apparent from the Chilean record over the same millennia.

We conclude that the trends of temperature and precipitation since ~43,000 yr BP, derived from the application of multivariate statistics to the pollen data at Taiquemó and Alerce, are generally representative of the Quaternary climates thus far established from some middle-latitude sites in the Southern Hemisphere. It should be possible to quantify further temperature and precipitation to demonstrate the degree of correspondence between land mass records through the last interglaciation. Fossil pollen records in this time range are available<sup>8,17</sup> but require quantification of the climatic variables from the pollen data.

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### Palaeomagnetic excursions, aborted reversals and transitional fields

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Recordings of palaeomagnetic excursions have revealed apparent field behaviour ranging from rather erratic directional mevements and loopings<sup>1-4</sup> to single, highly defined events<sup>5</sup> Excursions of the latter variety show what seems to be a rapid change in direction such that the path of the associated virtual geomagnetic poles (VGPs) is well constrained in longitude. Such behaviour is not unlike that observed at the onset of some recorded polarity transitions 7-9. Indeed, it has been suggested that excursions may occur during unsuccessful, or aborted, reversals2.5-7,10. We show here that available records of palaeomagnetic excursions, together with our present understanding of field behaviour associated with geomagnetic reversals, strongly support this hypothesis.

The ultimate question is: is it reasonable to consider at least some identifiable palaeomagnetic excursions to be records of aborted reversals and, therefore, relevant to our investigations of transitional field behaviour? If so, such data would provide a second major source of palaeomagnetic information to further our understanding of transitional field configurations and geomagnetic reversal.

It is first necessary to identify those palaeomagnetic recordings of claimed excursions which are the most likely to display accurately geomagnetic behaviour. As it is very important that the derived set of acceptable excursion records is not contaminated by spurious, inaccurate, or deceptive data, cautious and stringent criteria for acceptability must be imposed. For example, palaeomagnetic excursion data obtained from sediments will not be considered here, although they may include one or more reliable recordings (for example, ref. 4) Decause of the controversies and uncertainties surrounding many sediment-recorded excursions<sup>3,11</sup>. Similarly, excursion data obtained from sites at high latitude will not be considered. Exclusion of Icelandic data is particularly justified, given the relatively close proximity of the recording site to the geographical pole. The effects of secular variation at such high latitudes are known to be more pronounced and, hence tend to conceal systematic field behaviour related to excursion events. This may be the case with regard to Icelandic transition data12

Given the above arguments, to be considered acceptable a palaeomagnetic excursion record must: (1) be obtained from igneous rocks; (2) seem to be reliably determined, given the techniques used and results obtained; (3) contain a minimum of three successive data points (such as lava flows) associated with low-latitude (<45°) VGPs; (4) show that the intermediate directions were recorded during a clearly defined polarity state; and (5) be associated with a low or mid-latitude site.

Four excursion records satisfy the above criteria and, each is associated with a sequence of basalt lavas ranging from mid-Tertiary to Pleistocene. Table 1 lists the site description, age and relevant palaeomagnetic data for each. Note that all four of these acceptable excursion records show some longitudinal confinement of the low-latitude VGPs. As mentioned elsewhere, such behaviour is a common characteristic of palaeomagnetic recordings of Cenozoic polarity transitions

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		Table 1	Excursion dat	a			
Site location (ref.)	Coordinates	Full polarity sense	No. of low latitude VGPs	VGP path location relative to site*	Meridional band width†	Age	Symbol
Oahu, Hawaii (5, 18) Amsterdam Island (6) Nandewar Volcano,	21.5 °N 201.8 °E 37.8 °S 77.5 °E	Reverse Normal	3 5‡	+1.7 (Near) -150.7 (Far)	12.6 16.5	~3 Myr Brunhes	<b>♦</b>
Australia (19)  Liverpool Volcano,	30.3 °S 150.2 °E	Normal	5§	+161.9 (Far)	22.4	17.5 ±0.3 Myr	•
Australia	31.7 °S 150.2 °E	Reverse	3§	-3.6 (Near)	68.8	33.7 ±0.7 Myr	•

<sup>\*</sup> Mean angular longitudinal distance in degrees of low-latitude VGPs from site meridian (+) east of site, (-) west of site.

However, before comparing further these excursion data with those associated with successful field reversals, I shall briefly summarize our present knowledge regarding directional behaviour during field reversals.

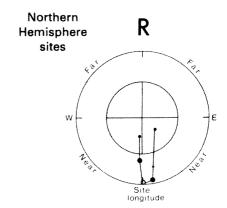
Recently much has been learned about the morphology of transition fields9. For example, comparison of palaeomagnetic records of the last reversal has shown the intermediate field to be predominantly non-dipolar 7,13,14. Moreover, VGP paths corresponding to this, as well as most other recorded Cenozoic polarity transitions, show a spatial dependence on the locality of the recording site 15. In particular, intermediate VGPs associated with reverse-to-normal  $(R \rightarrow N)$  transitions from sites in the Northern Hemisphere are found almost exclusively on the hemisphere centred about the respective site longitude 12.15, the so-called near side. Thus the non-dipolar component(s) controlling transition fields are axisymmetric and of low order<sup>12,15</sup>. However, our knowledge concerning this site dependence is incomplete. A more thorough understanding of transitional field geometries, as well as recognition of particular systematics associated with reversal in the core, require  $N \rightarrow R$ records from the Northern Hemisphere as well as records of both transition senses from the Southern Hemisphere<sup>12</sup>. At present the latter hardly exist9.

Two of the four acceptable excursion records correspond to events during normal polarity of the field and three of the recordings were obtained from sites at mid-southern latitudes. Hence, provided they are indeed representative of field behaviour during initiation of a reversal, these excursion records will greatly improve the existing polarity transition data set. Furthermore, provided the corresponding VGP behaviour is unambiguous, the combined successful/aborted reversal data may be sufficient to distinguish the predominant axisymmetric field geometry during transitions. Figure 1 shows the VGP data relative to site longitude, while taking into account the full polarity state of the field at the time of occurrence as well as the hemisphere of the recording site.

Note that the single record obtained from northern latitudes is associated with reverse polarity. Hence, this record serves to test further whether such field behaviour is the result of an unsuccessful reversal attempt. Comparison of the corresponding VGP path (Fig. 1, upper right) with existing  $R \rightarrow N$  transition data<sup>9</sup> strongly points to the affirmative. Indeed, this excursion path from Oahu, Hawaii is dramatically near sided with intermediate VGPs which closely straddle the site meridian. This finding, together with the weak relative remanence intensities associated with the excursion<sup>5</sup>, further justify the claim that aborted reversals exist in the palaeomagnetic record and, moreover, are

The remaining excursion data displayed in Fig. 1 correspond to records obtained from sites in the Southern Hemisphere. As with the Northern Hemisphere record, these three VGP paths are clearly found to be either near sided or far sided. In fact, it is

the single record corresponding to a reserve polarity excursion (Fig. 1, lower right) which is near sided while the two normal polarity excursions (Fig. 1, lower left) are far sided. Hence, all excursions which qualify as possible aborted reversals show intermediate VGP behaviour consistent with the finding in regard to transitional field geometries that non-dipole axisymmetric component(s) predominate. Moreover, the clear categorization of near-sided and far-sided paths by site locality and polarity state (during excursion) suggests that certain systema-



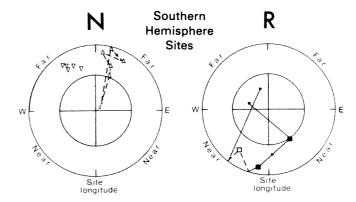


Fig. 1 Polar stereographic projections of VGPs from data sets listed in Table 1 and plotted with respect to site longitude. Each excursion is plotted separately depending on the hemisphere of the recording site and the full polarity state (N, normal; R, reverse) at the time of occurrence. Circles representing 45° latitude and the Equator are drawn in each case. Open and solid symbols (see also Table 1) represent Northern Hemisphere and Southern Hemisphere VGPs respectively. The Amsterdam Island data are shown without a path as a strict succession of flows was not presented in the literature<sup>6</sup>

<sup>†</sup> Width of meridional band containing all low-latitude VGPs.

<sup>‡</sup> Three of which are successive flows.

<sup>§</sup> VGPs calculated with respect to a location for Australia appropriate to the age of the sequence.

To be published elsewhere.

tics of the aborted reversal process exist. In fact, when these excursion data are combined with available detailed transition records9, a consistent set of VGP characteristics is obtained. Independent of the hemisphere in which the records were obtained, reverse polarity excursions and  $R \rightarrow N$  transitions are associated with near-sided VGP behaviour whereas normal polarity excursions and N -> R transitions are associated with far-sided VGP behaviour. These findings, listed by Hoffman and Fuller<sup>12</sup> as one possible regime, correspond to a predominantly quadrupolar  $(g_2^0)$  intermediate field geometry<sup>8</sup>

The finding that the VGP systematics depend on the sense of reversal supports the hypothesis that transitional fields are a result of the configurational characteristics of the reversal process in the core <sup>12–15</sup>. Moreover, these data are consistent with the idea that reversals have their origin in the Southern Hemisphere of the core. The hypothesis that a significant long-term standing (non-reversing) field exists during reversal attempts 16.17 is not supported, although a stationary field of short duration7 cannot be excluded.

Thus four records of palaeomagnetic excursions from low- to mid-latitude sites satisfy stringent criteria for reliability. Although few, these recordings display corresponding VGP behaviour which is constrained in longitude. Moreover, for each case, the path locality of the intermediate VGPs is unambiguously either near sided or far sided with respect to the longitude of the recording site. The single acceptable reverse polarity excursion from the Northern Hemisphere displays field behaviour consistent with that observed during R + N transitions also recorded at northern latitudes. These findings support the hypothesis that at least some geomagnetic excursions are aborted reversals and therefore the palaeofield variations derived from such records can be interpreted as transitional field behaviour. The intermediate VGP data (associated with the four acceptable excursion records), together with those from successful reversals, support the hypothesis that the controlling axisymmetric component of transition fields is quadrupolar. They also support the idea that the geomagnetic reversal process initiates in the Southern Hemisphere of the core. Why such an apparently symmetric entity as the Earth's outer core should be associated with an asymmetric reversal initiation process is debatable. The hypothesis of a long-term stationary, non-reversing field which controls the geometry during polarity transition attempts is not supported by these data. It remains to be seen whether additional palaeomagnetic records of either successful or aborted reversals will continue to support these conclusions.

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### Lower Proterozoic arc-microcontinent collisional tectonics in the western Churchill Province

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Arguments favouring evolution of the 'Hudsonian' orogen of the Churchill Province through plate tectonic processes generally supported by recent work in Saskatchewan and Manitoba-11. Palaeomagnetic data do not preclude operation of the Wilson cycle in this region during the Lower Proterozoic 12-15, At present, however, subduction-related satures in the Churchill Province are unconvincingly documented. Even the Thompson Belt ('Nelson Front'), forming part of the postulated suture between the Superior Province craton and the western Churchill mobile belt (Fig. 1), is markedly oblique to major volcanoplutonic and other lithostructural belts of the western Churchill and is thus unlikely to be a simple collisional junction. Orogens formed at consuming plate margins are likely to incorporate pre-terminal, arc-related sutures, variably obscured by later events16. Previous proposals for location of such intraorogenic sutures within the western Churchill belt, predicated largely on geophysical evidence17, are unsupported by recent geological investigations5. However, on the basis of recent fieldwork, I now outline a strong geological case for intraorogenic collisional suturing and microplate interaction in south-east parts of the Churchill Province in Saskatchewan.

Six major lithotectonic elements can be distinguished (Fig. 1). (1) The Rottenstone-La Ronge Magmatic Belt, developed at the south-east margin of continental crustal terrain, represented by the Cree Lake Zone<sup>5</sup>, is a Hudsonian 'Cordilleran-type' arc massif<sup>6,10</sup>. Most of the belt comprises the plutonic core of the arc: subordinate calc-alkaline, arc-type (Watters, personal communication) volcanics-volcaniclastics are preserved mainly in south-east parts of the belt. There is markedly asymmetric distribution of plutonic rock types 10, with dominant quartz monzonite-granite-granodiorite in the north-west and calcalkaline quartz diorite-tonalite-granodiorite in the south-east.

(2) The eastern La Ronge domain, a metasedimentary belt up to 20 km wide, mostly comprises quartz-plagioclase-biotite psammites and semipelites interlayered with, and transitional to, calcareous hornblende/diopside psammites, hornblende gneisses and amphibolites 18,19. Pebbly psammites and immature polymictic conglomerates form thin layers up to major units in, and locally transitional to, both psammites and hornblende gneisses. These rocks are interpreted as volcanogenic proximal greywackes, volcaniclastic-epiclastic sediments with minor lavas, and submarine fan deposits, derived from the Rottenstone-La Ronge arc. Both arc volcanics and derived sediments are stratigraphically overlain by locally conglomeratic arkoses preserved within an overturned disrupted synform on the western margin of the sedimentary belt.

The domain is affected by at least two deformation events which produced coplanar, north-east-trending isoclinal folds, foliation, limb-sliding and thrust stacking. These structures are deformed by open folding about north-trending axial surfaces. Strain indicators suggest extreme flattening normal to early foliation and fold axial surfaces, with very high extensional strains in the downdip direction.

On the basis of location, lithelogy and deformation, the eastern La Ronge domain is interpreted as the severely telescoped remnants of a forearc basin.

(3) Most of the Glennie Lake domain comprises early quartz dioritic-granodioritic orthogneisses and later foliated to undeformed dioritic to granitic plutons. Subordinate supracrustals occupy narrow arcuate belts within the orthogneisses: in the north-west they are in part continuous with, and similar to, supracrustals of the eastern La Ronge domain; elsewhere they include more abundant volcanics.

The domain has distinctive structural character. Early orthogneisses and supracrustals possess initially flat-lying gneissic foliation, isoclinal folding and lineation resulting from at least two deformation events. In the north-west, major recumbent folds can be correlated with more steeply overturned  $D_2$  isoclines of the Eastern La Ronge domain. These nappes are refolded by large wavelength, open to tight, north-trending  $D_3$ , and north-east-trending  $D_4$  folds, producing type 1 interference patterns. Differences in lithological character and structural succession in adjacent supracrustal zones  $^{20}$  may result from juxtaposition of autochthonous and allochthonous elements.

Most documented thermotectonic and plutonic events in the Glennie Lake domain are probably Hudsonian: limited Rb/Sr whole-rock dating (K. Bell and J. Blenkinsop, personal communication) has to date yielded no pre-Hudsonian ages. Nonetheless, lithostructural character and the nature of domain boundaries indicate that the domain possesses fundamentally different basic crustal structure and evolution compared with adjacent domains.

The south-west part of the La Ronge-Glennie Lake domain junction is defined by the 2-4 km wide Stanley Shear Zone<sup>18</sup> which incorporates submylonitic and blastomylonitic gneisses interpreted, on structural and metamorphic evidence, to have developed early in local thermotectonism. To the north-east, this early 'straight belt' disappears and the Stanley Zone is marked only by late dextral strike-slip faults, across which broad

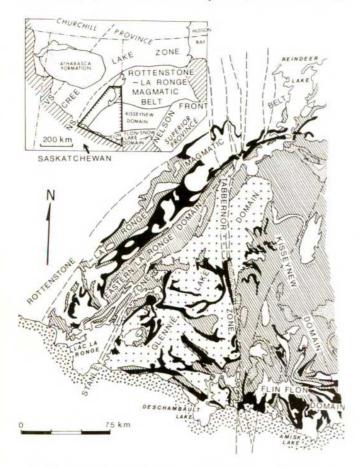


Fig. 1 Geology of the southeastern part of the Churchill Province in northern Saskatchewan (location indicated by heavy, hatched lines in inset). Solid black area, undifferentiated metavolcanics; ⊠, mainly volcanogenic metasediments; ⊠, meta-arkoses; | ++ |, undifferentiated granitoids of Glennie Lake domain and Hanson Lake area; unshaded area, quartz dioritic to granitic plutons of other domains; | -- |, Phanerozoic cover (oblique line in inset). Dashed lines indicate fault and shear zones. VS (inset), Virgin River Shear Zone; NS (inset), Needle Falls Shear Zone.

continuity of lithology and structural trends occur. Even here, however, the domain junction is marked by major changes in fold style and attitude.

(4) South of Reindeer Lake, the Tabbernor Zone is a narrow 'straight belt' with a complex history dating from early- and possibly pre-Hudsonian time. It includes blastomylonitic gneisses formed early in Hudsonian thermotectonism<sup>21,22</sup>, tightly appressed northerly-trending folds and syn- to post-metamorphic faults. The zone is marked by a pronounced metamorphic gradient<sup>21,23</sup>. The southern part of the zone was thus a fundamental crustal dislocation active throughout Hudsonian orogeny.

To the north, the Tabbernor Zone changes abruptly in character and documented history as it extends across the Rottenstone–La Ronge belt, marked most obviously by splaying of the shear zone into a broader, diffuse set of fractures. The latter are associated with late Hudsonian open northerly folds in the La Ronge domain but these disappear farther north and the Tabbernor system is thereafter characterized only by brittle faults with sinistral strike-slip displacement. All early Hudsonian phenomena disappear as the Tabbernor Zone leaves the Glennie Lake–Kisseynew terrain.

(5) The Kisseynew domain mostly comprises highly migmatized, proximal to distal volcanogenic metagreywacke turbidites, subordinate metavolcanics and arkoses<sup>7,24</sup>. Interdigitating facies relations with supracrustals of the La Ronge-Lynn Lake and Flin Flon-Snow Lake volcanoplutonic arcs are well documented and the 'Kisseynew Gneisses' are generally interpreted as being deposited in part contemporaneously with arc development. Original continuity between the northern Kisseynew terrain and the eastern La Ronge forearc seems certain.

Most granitoids of the Kisseynew domain are locally derived anatectites. Major Hudsonian plutons are uncommon, except adjacent to the La Ronge and Flin Flon belts. Older 'basement' is recorded only immediately east of the Tabbernor Zone, in the south-west part of the domain: the Sahli Granite is dated at  $2,430\pm30$  Myr (Rb/Sr whole rock isochron)<sup>25</sup> and volcanics and granitic rocks in the Hanson Lake area at 2,521 and  $2,446\pm16$  Myr (Rb/Sr whole rock isochrons)<sup>26</sup>. Rocks of the latter area are akin to those in adjacent parts of the Glennie Lake domain and provide tenuous geochronological indication of possibly extensive older continental basement in the latter.

(6) The Flin Flon domain (and its extension, the Snow Lake belt of Manitoba) is a well documented Lower Proterozoic arc complex<sup>27-29</sup> similar in most respects to the Rottenstone-La Ronge Magmatic Belt. Age data indicate that the two arcs are essentially coeval but suggest the Flin Flon arc could be slightly younger<sup>30</sup>.

The Glennie Lake domain has previously been interpreted, speculatively, as incorporating pre-Hudsonian continental crust<sup>6,11,18</sup>. While this is unconfirmed by radiometric data the domain possesses distinctive lithostructural style and is bounded largely by major tectonic discontinuities. Metamorphic data strongly emphasize domainal contrasts and support interpretation of the Tabbernor Zone as a fundamental crustal feature. Interpretation of the Glennie Lake domain as possessing radically different pre-Hudsonian crustal character, relative to adjacent domains, provides a possible explanation of regional geology and gross structural geometry, and accords with a general model for Hudsonian evolution in the southeastern Saskatchewan–Manitoba Shield.

It is thus argued that in early- to mid-Hudsonian time the Glennie Lake domain formed a microcontinental block within a dominantly oceanic lithospheric plate which was subducting beneath the Rottenstone–La Ronge arc along a consuming plate margin located south-east of the eastern la Ronge–northern Kisseynew terrains (Fig. 2a). This resulted in collision of the Glennie Lake block with the eastern La Ronge forearc and ensuing arc–microcontinent suture (Fig. 2b). The collision produced extreme telescoping of the forearc prism, locally leading to almost complete squeezing out, at the present erosion

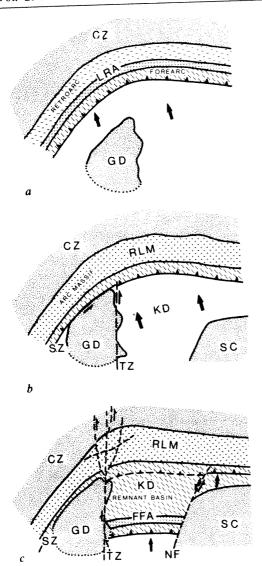


Fig. 2 Possible tectonic evolution of the south-east Churchill Province in Saskatchewan and Manitoba. a,  $(\sim 1.950-$ 1,900 Myr?): early Hudsonian development of subduction zone and La Ronge-Lynn Lake volcanic arc marginal to Cree Lake continental margin. The Glennie Lake domain is an 'outboard' microcontinent within a predominantly oceanic subducting plate. b, (~1,900-1,870 Myr?): collision of Glennie Lake domain with eastern La Ronge forearc and initial telescoping of the forearc prism. Uncoupling occurs between the impeded Glennie Lake microplate and still subducting Kisseynew microplate with initiation of the southern Tabbernor Zone. The arc is uplifted and stripped to form a major Cordilleran-type arc massif, the Rottenstone-La Ronge Magmatic Belt. The Flin Flon-Snow Lake arc may be initiated by migration of subduction site southwards between the Tabbernor and Thompson 'transforms' or may already have been active in the outboard part of the southern plate.  $c_1 (\sim 1,860 \text{ Myr?})$ : the Glennie Lake domain is sutured to the arc massif with immense telescoping of the forearc and possible transcurrent reactivation along the suture. Subduction north of the Kisseynew domain has ceased and the suture buried beneath sediment influx. The Kisseynew subplate becomes an interarc remnant basin receiving sediments both from north and south. Differential movements propagate the Tabbernor Zone northwards as a splaying wrench system. The Thompson Belt forms another transcurrent/transform junction, to the east of which incoming of the Superior continent leads to terminal collision along the northern part of the Nelson Front, followed by general compression, late folding, continuing propagation and reactivation of major fractures. Assigned dates are speculative, based on limited radiometric data. CZ, Cree Lake Zone; GD, Glennie Lake Domain; SC, Superior Craton; LRA, La Ronge-Lynn Lake Arc; FFA, Flin Flon-Snow Lake Arc; RLM, Rottenstone-La Ronge Magmatic Belt; KD, Kisseynew Domain; SZ, Stanley Zone; TZ, Tabbernor Zone; NF, Nelson Front (Thompson Belt).

level, of forearc sediments between the arc massif and microcontinent. Deformation and indicated bulk strain in the forearc belt are consistent with this interpretation. Recumbent fold/thrust sheets were driven southeastwards out of the forearc across the advancing microcontinent. In part, such nappes may include thrust slices from the leading edge of the microcontinent. The Stanley Shear Zone is thought to represent, not the actual collision suture, which is presumably overridden, but the main sole zone of detachment of allochthonous sheets moving over the Glennie Lake block. Farther north this detachment zone is folded and plunges beneath the allochthon.

Further deformation in the Glennie Lake domain, accompanying final collision stages (and possibly related also to new or continuing subduction to the east or south) resulted in major refolding of autochthonous basement and allochthonous cover, accompanied and outlasted by plutonism. Reactivation along the suture zone produced later faulting which extended into the overriding allochthon, thus generating the presently exposed north-east part of the Stanley fault system.

The Tabbernor Zone is interpreted as a transform junction initiated between the Glennie Lake microcontinent and the Kisseynew subplate (which may be underlain by predominantly oceanic lithosphere). Uncoupling along this zone is thought to have accompanied locking up of the Glennie Lake block in relation to still subducting lithosphere underlying the Kisseynew domain (Fig. 2c). Though the Tabbernor Zone generally conforms to the Glennie Lake domain junction it seems that prominences were detached as fragments within the dominantly oceanic subplate, as, for example, the Hanson Lake block.

The Kisseynew basin was filled with detritus from the La Ronge-Lynn Lake and Flin Flon ares, which may have been active simultaneously for a time: alternatively, the Flin Flon arc may be younger and result from rapid migration, or jump, of the subduction site along the transform junction. Resolution of these alternatives awaits more refined stratigraphic mapping and radiometric dating. The subduction zone related to the eastern part of the La Ronge-Lynn Lake arc is thought to be buried beneath Kisseynew basinal sediments.

The Flin Flon-Snow Lake arc is bounded by the Tabbernor Zone to the west and the Thompson Belt to the east: the latter is interpreted as another major transform, or transcurrent, junction between the Kisseynew subplate/remnant ocean basin and Superior continental craton. Northerly subduction beneath the arc is invoked to explain high grade metamorphism of the Kisseynew terrain, as compared with the southern Glennie Lake domain and Superior craton.

Stresses induced in the already crystalline Rottenstone-La Ronge arc massif and Cree Lake Zone, due to differential activity of segments of the subducting plate, are postulated to have caused northwards propagation of the Tabbernor Zone across the underthrust continental plate as a splaying wrench system. Further propagation and reactivation accompanied late-Hudsonian terminal collision and overall compression, producing sinistral displacements along the Tabbernor Zone and reactivation of other major discontinuities such as the Stanley Zone.

The proposed model accounts for hitherto unexplained differences in character of northern and southern parts of the Tabbernor Zone and its oblique orientation relative to other elements of the western Churchill Province. It provides a viable explanation of the 'abberrant' orientation of the Thompson Belt in plate tectonic modelling of the Hudsonian orogen.

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#### Volcanic components in pelitic sediments

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The origin of the clay minerals present in pelitic sediments has been widely debated. Weaver suggests that illite and interstratified illite/smectite (I/S) dominate. The association of illite with marine sediments<sup>2</sup> and the proposal<sup>3,4</sup> that illite forms from smectite (an expandable clay mineral sometimes referred to as montmorillonite) in the marine environment has also been discussed. Weaver<sup>5</sup> argues for a continental origin of illite, produced by weathering of K-feldspar. Others 6-9 have shown that smectite and I/S (20-50% illite) are converted to I/S of higher illite content (60-80%) in pelitic sediments by low-grade metamorphism. Per cent illite will always refer to the per cent illite in I/S. The designation should not be confused with the content of discrete illite or mica commonly present in these sediments. Here we present clay mineral and K-Ar data for Cretaceous marine shales from the Western Interior of North America demonstrating a volcanic origin for much of these sediments. This observation is correlated with eustatic sea-level rise, increased volcanic activity, sedimentary preservation of organic matter, and incorporated into a petrogenetic/tectonic model for pelitic sediments.

It has been shown 8-10 that Cretaceous marine sediments from the Western Interior of North America are primarily composed of random I/S11. In some areas, the clay mineral assemblages of these sediments have been converted to ordered I/S<sup>8,9</sup> and illite by low-grade metamorphism. Unpublished X-ray diffraction data of contact metamorphic Cretaceous clay mineral assemblages in the Spanish Peaks area, Colorado, clearly demonstrates that the I/S is converted to 100% illite. Fine-grained, vitreous, volcanic debris is generally accepted as the source of the smectite clay<sup>1,12</sup>. The presence and resulting mineralogy of numerous bentonites13 associated with these sediments supports this interpretation. I/S clay mineral data from 77 bentoniteshale sample pairs in the Cretaceous Mancos shale are shown in Fig. 1. Excellent agreement between the nature of interstratification of the sample pairs is indicated. Although the bentonites order before the shales, the per cent illite is generally higher in the shales, particularly for those sample pairs of random interstratification (Fig. 1). Schultz<sup>10</sup> has noted a similar discrepancy and proposed that this greater amount of illite in shales compared with bentonites may be related to longer marine residence times of volcanic debris and consequent alteration to random I/S in the resulting shales. High sedimentation rates of volcanic debris, resulting in the formation of

Table 1 K-Ar age determinations of clay size fractions from Cretaceous marine shales

Sample	Potassium-bearing clay mineral	Age (Myr)
MS921	Randomly interstratified illite/	98.3±4
MS86	smectite (I/S), 50% illite Discrete illite	(Upper Cretaceous) 2.3 ± 8 (Triassic)

distinct bentonite beds, have a much shorter marine residence time and are therefore essentially smectitic. Consequently, bentonite-clay alteration occurs primarily below the sedimentwater interface, and may explain the very low illite content of I/S in bentonites. We agree with this interpretation. However the similarity in clay mineralogy of non-marine and marine Cretaceous pelitic units may indicate a continental role in the formation of random I/S from volcanic debris (L. G. Schultz, personal communication). This point is important because the location of clay potassium adsorption, and consequent formation of illite, has major implications with regard to the chemical cycle and distribution of potassium in the lithosphere and hydrosphere. For this discussion, we contend that despite the discrepancy in illite contents of I/S, the gross similarity of the clay mineralogy of Cretaceous shales and bentonites, particularly with regard to the nature of interstratification, suggest a common origin, that is, the result of alteration of volcanic debris.

K-Ar investigations of pelitic sediments, which are primarily concerned with the dating of illite, the major potassium-bearing phase, have produced complex results14 that are partly due to the mixing of detrital illite derived from older lithologies and younger metamorphic illite<sup>15,16</sup>. Similarly, K-Ar interpretation of the Cretaceous shales is complicated by the presence of minor amounts of discrete illite in almost all the clay size fractions of the samples. However, a few shales were found to have clay size fractions composed of either random I/S or pure discrete illite. K-Ar determination of these samples (Table 1) indicate that the discrete illite (MS86) is probably detritus from an older source (213 Myr), that is, pre-Cretaceous, whereas the random I/S (MS921) yields approximately the correct stratigraphic age (98 Myr). Additional evidence is provided by the K-Ar investigation of Hoffman<sup>17</sup> on Missouri River sediments in which Cretaceous shales are the major stratigraphic units in the drainage basin. In this study, clay minerals from 11 sediment samples, essentially random I/S, yielded ages of 79-125 Myr averaging ~100 Myr. Bentonites, on the other hand, often contain no detrital sedimentary components. If the original smectite in bentonites is converted to ordered I/S, there is little doubt that the measured age records potassium fixation and

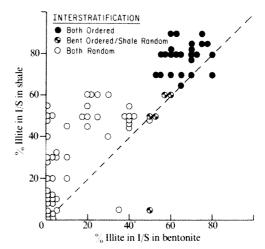


Fig. 1 Relationship between percentage illite in interstratified illite/smectite (I/S) from bentonite-shale sample (equivalents line (broken) is shown for comparison).

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consequent illite formation during burial or contact metamorphism 16. Five samples of such bentonite materials were dated by K-Ar methods, and produced ages between 62 and 40 Myr (ref. 18). The clay from shale that has the correct stratigraphic age (98.3 Myr) could be produced by a mixture of discrete illite and ordered I/S, however, no trace of either discrete illite or ordered I/S was resolved by X-ray diffraction. A simple calculation shows that sufficient 200-Myr material is needed to increase a 60-Myr age to 100 Myr producing a mixture in which discrete illite would have been clearly resolved in the diffraction pattern. We therefore conclude that the 98.3-Myr random I/S is a mineral phase whose potassium content was fixed at or near the time of Cretaceous sedimentation, hence supporting the contention that random I/S represents first-cycle sedimentary material resulting from the alteration of volcanic debris. Hoffman<sup>17</sup> drew similar conclusions from his age data.

The Cretaceous is an unusual period in several respects. The proliferation of epicontinental marine sediments due to a high stand of eustatic sea level during this period produced marine transgressive sedimentary sequences on almost all the continents19,20, including the invasion of marine depositional environments into the Western Interior of North America. Gilluly21 shows the difficulty in providing a detrital source area for the Cretaceous marine sediments of North America (volume =  $4 \times 10^6 \text{ km}^3$ ). Based on particle size and quantitative clay mineral analyses of the Cretaceous Pierre shales, I/S represents ~38% of the total sediment<sup>22</sup>. From a comparative study of coarser size fractions of the Pierre shales with those of bentonites Jones<sup>23</sup> estimates that  $\sim 40\%$  of these sediments are the result of pelagic deposition of aeolian transported volcanic debris. Therefore, the data indicate that approximately onethird to one-half of these sediments are of volcanic origin, possibly explaining the difficulty in reconciling their source from reworked older rocks. The influx of volcanic material into the Cretaceous marine environment was episodic, and at times completely dominated the sedimentation as evidenced by the presence of numerous bentonites. This form of sedimentation is characterized by relatively uniform sediment accumulation over much of the basin and is much different from that of fluvial deltaic systems that localize sediment in more restricted depositional environments. Petrologically, the Cretaceous shales have many features in common with black shales elsewhere. The depositional environments resulting in the formation of black shales are of great interest because of their association with hydrocarbon and some types of metallic ore deposits. Factors important in their formation are organic productivity, sedimentation rate, and rate of oxidation of organic matter before burial<sup>24,25</sup>. The volcanic-sedimentary episode may have had other geochemical consequences, such as the influx of chemical elements into the atmosphere and oceans from volcanic ash and gases. These factors may provide a conducive environment for the formation of black shales. Organic-rich pelagic sediments were preserved globally in various marine environments during the Cretaceous<sup>26,27</sup>. These 'anoxic events' have been correlated with the rise in eustatic level, increases in organic production and changes in global climate. Scholle and Arthur<sup>28</sup> have shown that the <sup>13</sup>C isotopic composition of Cretaceous carbonates increases from 120 to 80 Myr, reaching a maximum at 108 Myr. This increase in <sup>13</sup>C is correlated with the increased preservation of organic matter in these sediments. Other studies<sup>29,30</sup> have shown a dramatic 3-4-fold increase in the rate of seafloor spreading (up to 18 cm yr<sup>-1</sup>) during the mid-Cretaceous (110-85 Myr), and that there is a direct relationship between the rate of spreading and eustatic sea level. Kauffman<sup>31</sup> has noted a correlation between emplacement of calc-alkaline batholiths and bentonite frequency in marine sediments, suggesting a link with tectonic activity. McBirney<sup>32</sup> observed a relationship between marine transgressions and episode volcanic activity which tends to be synchronous over a broad region of the Earth. All the evidence suggests that these dynamic Earth processes are related. The record of high eustatic sea level would correspond to periods of higher volcanic activity, the debris from which

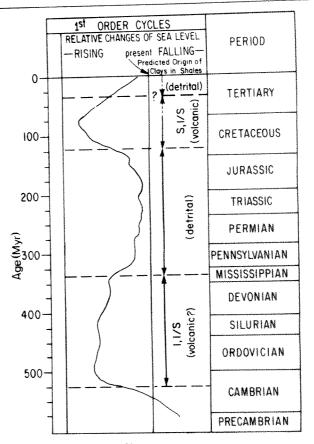


Fig. 2 Eustatic sea level<sup>20</sup> and clay petrology of pelitic sediments (mainly after ref. 1) throughout the Phanerozoic. I, illite; I/S, interstratified illite/smectite; S, smectite.

accumulates in the greatly expanded marine environment, readily altering to smectite and random I/S.

This discussion may be relevant to the origin of the bulk of clay minerals preserved in pelitic sediments. Are there any analogues to this form of sedimentation proposed for the Cretaceous? Some cases of the lower to mid-Palaeozoic systems of eastern North America are good candidates, given the proliferation of marine sedimentation, the widespread occurrence of Ordovician and Devonian bentonites, and the formation of black shales. In Weaver's classic work on the clay petrology of sediments, he notes that the dominant clay mineral present in pre-Upper Mississippian sediments is illite and I/S. Here again a discrepancy exists between the clay mineralogy of Palaeozoic bentonites, which are I/S, 80-90% illite, and shales which contain I/S that is generally 90% illite, and illite. In the Cretaceous sediments, I/S in the shales have a higher illite content than do the bentonites, and should this lag in illite content continue through higher grades of burial metamorphism, the I/S of shales would be expected to convert the 100% illite before the I/S of the bentonites. Given the relationship of eustatic sea level and volcanic activity, inspection of Fig. 2 predicts that much of the illite and I/S of these sediments may represent the accumulation of volcanic debris, originally in the orm of nearly pure smectite and random I/S, later modified by netamorphic conversion of smectite to illite. Periods of low eustatic sea level would be predicted to have a lower overall volcanic influx, allowing the reworked detrital influx to dominate. The abundance of pelitic sedimentary and metamorphic rocks, which make up more than half of the sedimentary record<sup>34</sup> in continental source areas, combined with the stability of phyllosilicates in aqueous systems are in part responsible for their presence in detrital clay mineral assemblages. Therefore, incorporation of older, volcanically derived clay into later depositional environments is a common occurrence. The Clay mineral assemblages of the Upper Palaeozoic and Lower to Middle Mesozoic<sup>1,2</sup>, as well as Recent marine sediments<sup>33</sup>, are in fact, highly variable in composition and lack

the uniformity of the lower to mid-Palaeozoic and most of the Cretaceous.

We have tried to rationalize many different facts and hypotheses for the Cretaceous sedimentation in North America. A major marine transgression was accompanied perhaps by increased volcanic activity at the subduction zones. Clay mineral and K-Ar data from Cretaceous shales suggest that the smectite and I/S which are major components in these sediments are derived primarily from volcanic debris. Thus one model exists that explains the aqueous environment for the accumulation of a sedimentary record, and the volcanic sources to fill it. Illite, I/S and smectite are the dominant clay minerals in pelitic sediments. The similarity between clay mineral assemblages of Lower to Middle Palaeozoic marine shales with the inferred mineralogy of Cretaceous marine shales after deep and prolonged burial suggests that the Cretaceous model described above may be general, and that the derivation of smectite and random I/S from volcanic debris and its later metamorphic conversion to ordered I/S and illite is a major reaction sequence affecting the clay petrology of pelitic sediments.

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#### Sinian microfossils from south-west China

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The microfossil assemblages described here are preserved in cherts from the Yunnan Province, south-west China. They are the first chert-facies microfossils to be discovered in the Wangjiawan and Meishucun stratigraphical sequences which are regarded by the International Cambrian/Precambrian Boundary Working Group1 as possible 'candidates' for the stratotype section of the Cambrian/Precambrian boundary.

The rock samples in which the microfossils were found were collected from the middle member of the Dengying Formation and from the lower member of the Meishucun Formation of the Sinian 'system' the Wangjiawan and Meishucun sections (Fig. 1). The carbonaceous rocks of the upper member of the Meishucun Formation at both sections (W. and M.) have been dated to  $603 \pm 31$  Myr and  $612 \pm 36$  Myr respectively (by Rb-Sr isochron dating where  $\lambda^{87}$ Rb is 0.142 yr<sup>-1</sup>)<sup>1</sup>. The fossiliferous area of the Meishucun Formation in the Wangjiawan section is more than 60 m below the oldest trilobite-bearing beds of the Lower Cambrian and about 15-20 m above the lowest occurrence of small shelly fossils. The stratigraphical position in the Meishucun section is more than 50 m below the oldest trilobite-bearing beds. The fossiliferous bed of the Dengying Formation of the Wangjiawan section is more than 100 m below the lowest beds containing small shelly fossils. The fossiliferous rocks consist of non-stromatolitic chert laminae or lensed bodies (nodules) which form intercalated beds in dolomite or dolomitic fine-grain sandstone.

The type of microfossil preserved in the cherts of the Wangjiawan section is the same as that previously reported for Precambrian deposits; however, the microfossils from the cherts of the Meishucun section are strongly mineralized, light in colour and partially or totally lacking in organic matter, so that they can only be considered as fossils on the basis of their apparent biological nature. Secondary silification may be a good process for preserving microfossils originally mineralized after

The Dengying and Meishucun microfossil assemblages are morphologically of three forms-spheroids, trichomes and sheath-like filaments. Most of the coccoids and filamentous forms are well preserved. Although most of the fossils are degraded forms and lack detailed structure, their morphology, size range and the cell structures occurring randomly in some of the samples enable their classification either as a previously described Precambrian taxon or as a new genus or species.

Among the coccoids, the colonies shown in Fig. 2f are assigned to Clonophycus Oehler<sup>2</sup>, which have been compared with large cells of eukaryotic green or red algae; those in Fig. 2e, g are assigned to Myxococcoides kingii Muir<sup>3</sup>; and the samples shown in Fig. 2n may be a new taxon of non-ensheathed colonies composed of unicells which occur in individual spheroids, in pairs or in planar tetrads, and are 7-22 µm in diameter (commonly 11-16 µm), with a psilate surface texture, but no sheath or organic matrix. Both the known taxa can be compared with members of the Recent Chroococcaceae. Samples of solitary unicells (Fig. 2a-c) are assigned to Myxo-

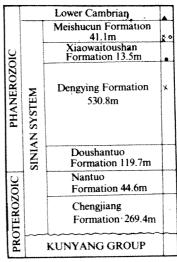


Fig. 1 Stratigraphy of the Sinian System in Yunnan Province, China, showing the occurrence of fossiliferous chert samples from the Wangjiawan section (X), the Meishucun section (O), and the oldest trilobite bed (A) and lowest bed containing small shelly fossils ( ) in Wangjiawan section.

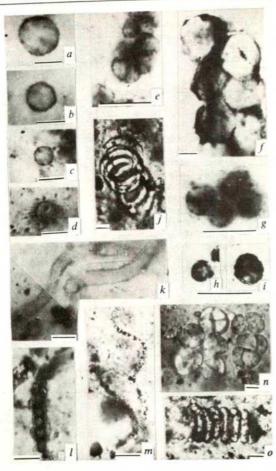


Fig. 2 Optical photomicrographs of the Sinian fossils discovered in chert thin sections from the Meishucun Formation (a-e, g-i, k) and the Dengying Formation (f) of the Wangjiawan section, and from the Meishucun Formation of the Meishucun section (j, l-o). Scale bar,  $10~\mu m$ .

coccoides grandis Horodyski & Donaldson<sup>4</sup>. Spheroidal envelopes (or round flattened membranes) (Fig. 2h, i), which closely resemble acritarcha fossils obtained by simple palynological methods from Precambrian sediments, are of the taxum *Protosphaeridium* Timofeev<sup>5</sup>. Several coccoidal unicells with radial setaceous spines (Fig. 2d) were discovered in the cherts of the Meishucun Formation from the Wangjiawan section; they resemble members of acritarcha *Baltisphaeridium* discovered in Russian Cambrian sediments by macerating rock<sup>6</sup>, and are 6.5–7 μm in diameter, with spines <0.5 μm wide and 2.5–5 μm long.

In some thin sections of chert from the Meishucun Formation of the Wangjiawan section only one kind of non-septate filament (Fig. 2k) has been discovered which has been assigned to Eomycetopsis robusta Schopf emend Knoll & Golubic<sup>7</sup>. This taxum includes structures resembling the empty sheath of cyanophitic or bacterial trichomes. Other sections from the same formation contain extensively degraded non-septate filaments of this taxum (Fig. 2m) together with an abundance of several other kinds of filament. Those shown in Fig. 3h, i are of the species Cyanonema inflatum Oehler<sup>2</sup>; comparable with the recently discovered member of Oscillatoriaceae; the coiled filaments shown in Fig. 2j, o are assigned to the Obruchevella parva Reitlinger<sup>8</sup> and comparable with recently discovered cyanophitic blue-green alga Spirulina. The sample illustrated in Figs 2l and 3a-g, j may be a new taxon.

The filaments shown in Fig. 3f, g appear to be unbranched, uniseriate, septate and multicellular and resemble Cyanonema inflatum except for the occurrence of a thin sheath and some larger cells that may be heterocysts (?) typical of the Nostocaceae. The specimens shown in Fig. 3e seem to be degraded

trichomes of uniseriate, septate, multicellular filamentous algae, although the filaments have indistinct outlines; the septate filaments can also be seen in the well preserved larger cells (which may be heterocysts) distributed along the filament. The trichomes are  $\sim 3-4~\mu m$  in diameter, and the larger cells 4–5  $\mu m$  wide and 4.5–6.5  $\mu m$  long; each of the larger cells contains a black body, 1–2  $\mu m$  in diameter, irregular or oval in shape and comprised of degraded cellular matter. The specimens are similar to members of the genus  $Veteronostocale^9$ , which has been compared with recently found Nostocaceae; however, they are unnamed here because of their poor state of preservation.

The larger filaments dicovered are shown in Fig. 3a-d, j; all resemble recently discovered members of Oscillatoriaceae, being unbranched, uniseriate, septate and multicellular or nonseptate tubular, disk-like trichomes with a distinct sheath. Figure 3a shows an ensheathed trichome; such trichomes occur as a coiled filament 26-30 µm in diameter, as a round coiled body 250 μm in diameter, or as disk-like medial cells, 3-8 μm long and 26-28 µm wide. The samples may represent fragments of a larger member of the Obruchevella, or a new taxon not yet described, and are tentatively named here Circulinema jinningense gen. et sp. nov. on the basis of their preserved state. The coiled filaments shown in Fig. 3b-d resemble C. jinningense except for their lack of internal cells; they occur in non-septate form are 19-21 µm in diameter and coil to give bodies 73-140 µm in diameter; this type of coiled filament may represent the empty sheath of C. jinningense or the degraded form of the latter which have few or no internal cells because of degradation. Figure 3j shows a fragment of a larger member of the Oscillatoriaceae, 27-28 µm in diameter, with a distinct sheath and disk-like medial cells.

The Sinian microfossils described here are of interest in Precambrian microbial study because: (1) although other Pre-



Fig. 3 Optical photomicrographs of the Sinian fossils discovered in chert thin sections from the Meishucun Formation of the Meishucun section. Scale bar, 10 μm.

cambrian microfossils have been described in China, they are mainly acritarcha fossils macerated from non-chert rock samples; few microfossils from cherts of both stromatolitic and non-stromatolitic types have been described10. The assemblages described here are apparently the best Sinian assemblages from south-west China reported to date. (2) The Sinian assemblages occur near the Cambrian/Precambrian boundary, and the abundance of the wider filaments and some stratigraphically definite taxa, such as the Obruchevella parva reported only from sediments near the Upper Proterozoic/Lower Phanerozoic boundary8, may allow assignment of a late Proterozoic age for the sediments. (3) Although the small and ensheathed colonial unicells and filamentous fossils dominate in the described formations and suggest cyanophytic affinity with Chroococcaceae, Oscillatoriaceae and Nostocaceae, the large coccoids are larger than most of the cyanophytic taxa and may be eukaryotic like the members of Chlorophyceae or Rhodophyceae discovered in stromatolitic cherts. This suggests that the stromatolite-building algae are taxonomically similar to the dominant benthic algae inhabiting the supra-, sub- and intertidal late Precambrian environment, and it is of interest to know where and how these microorganisms build the stromatolites. (4) The better preservation of the mineralized microfossils in cherts of the Meishucun Formation in the Meishucun section may indicate the importance of secondary mineralization as a means of preservation and that this kind of microfossil is probably filled by collophanite before replacement by apatite.

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#### Tree remains in a North York Moors peat profile

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It has been postulated1,2 that in the southern Pennines above 350-400 m, burning of the vegetation during Mesolithic time prevented the growth of trees, and that this continued during Neolithic time, although it was suggested that some recolonization of forest took place when agriculture made it possible to diminish the impact of hunters on wild animal food resources and on these uplands. Here we present analogous evidence but come to a slightly different conclusion which may be due to regional differences in prehistoric settlement. Peat profiles such as we describe here are uncommon in the region and so the phenomena may have been spatially restricted.

Table 1 Stratigraphical units at Bonfield Gill Head

***************************************			
Unit letter*	Horizons occupied (cm)	Peat type	Wood and macroscopic charcoal contents*
1	0-20	Sphagnum peat	No charcoal
k	20-40	Well humified peat with some remains of Eriophorum	No charcoal above 41 cm, Betula stump at 38-41 cm
j	40-52	Less well humified	
	(Ulmus	Eriophorum peat,	
	decline	some Ericaceae	
	at 41 cm)	twigs	
i	52-54	Wood layer and amorphous peat	Betula wood: bark, branches and twigs
h	54–58	Charcoal and amorphous peat	Large pieces of charcoal
g	58-70	Eriophorum peat	
g f	70-77	Well humified peat and wood	Small wood pieces (up to 10×5 cm)
e	77–81	Peat and charcoal	Charcoal mostly macroscopic but 'soot' also present
d	81-90	Wood fragments and peat	Betula fragments
c	90-97	Minero-organic amorphous peat	
b	97-105	Sand + wood and bark fragments	Tiny pieces of woody material
a	105 →	White clay	*

See Fig. 1.

We examined the peat profile from the head of Bonfield Gill (grid ref. SE 598958: 346m altitude) which runs east-west across Bilsdale Moor in an area of patchy blanket peat which has eroded in places to reveal stumps and trunks of sub-fossil trees (Betula and Quercus spp.) of a substantial size; the remnants of forest rather than scrub are suggested by trunks 0.6-1.0 m in diameter with roots spreading a further 1.25 m to each side. Investigation of the basal 100 cm of a typical profile of 2-m depth of organic material reveals several stratigraphical units (Table 1 and Fig. 1). The key elements in the stratigraphy are the presence of wood in several layers and the occurrence of charcoal and 'soot' (microscopic charcoal fragments) particles throughout. An estimate of the frequency of the microscopic particles at different horizons is given in Fig. 2. Macroscopic charcoal occurrence is indicated in Table 1. At 38-41 cm there is the base of a well preserved Betula tree stump whose remains and attitude leave no doubt that it is in situ, together with wood fragments of Quercus sp. Above the stump we find fibrous monocotyledonous peats and Sphagnum peat typical of blanket mires in upland Britain. This stratigraphy accords with the southern Pennine profiles in recording tree growth after a period of peat accumulation. In the lower peats (52-54 cm, 70-77 cm) the wood remains comprise fragments of birch bark and birch twigs, some of which are so large that they cannot have travelled far, and may represent in situ woodland.

Results from the pollen analysis of the basal 100 cm of the profile are displayed as Fig. 1, pollen frequencies being expressed as percentages of total land pollen. The strata described in the stratigraphic column are explained in Table 1, and above this sampled section lay a further metre of humified Sphagnum peat. In Fig. 1 certain taxa have been assembled into groups in accordance with their ecological affinities. 'Quercetum Mixtum' includes Quercus, Ulmus and Tilia. 'Heliophyte Shrubs' incorporates light-demanding woody taxa which respond favourably to forest canopy opening; Prunus, Sorbus, Salix and Fraxinus. 'Ruderals' includes herbs considered to be colonizers of waste or freshly cleared ground. Zonation of the pollen diagram is by a scheme of local forest clearance stages (FCS) of which three types are defined. Interference (I) phases recording

<sup>\*</sup> For 'soot' see Fig. 2.

low-intensity earlier clearance, clearance (C) phases recording higher-intensity later clearance and regeneration (R) phases during which forest clearance is considered not to be taking place. Radiocarbon dates are given in Table 2 and Fig. 1.

Correlation of the pollen and stratigraphical data reveals several interesting features. First, the relationship of the tree stump to a decline of *Ulmus* pollen: the other pollen frequencies, the <sup>14</sup>C date and comparison with other North York Moors pollen profiles suggest strongly that this is the *Ulmus* decline of western Europe, usually dated in this region to ~4,750 yr b.p. (ref. 3). If we accept this marker horizon and agree also that it is the spoor of the incursion of people of Neolithic culture, then the tree stump marks the end of Mesolithic time. Inspection of the pollen frequency curves for the 40–100 cm section of the profile reveals evidence corroborative of the interference with the vegetation suggested, particularly by the charcoal, in Table 1. This evidence takes the form of 'interference phases' of which three are recognized (I1, I2 and I3 on Fig. 1) and two of which are <sup>14</sup>C dated to the 5th millenium b.p.

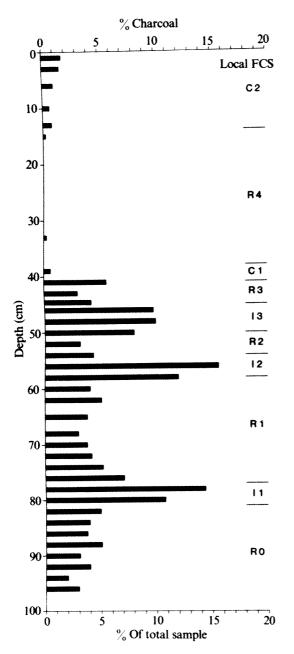


Fig. 1 Selective pollen diagram from Bonfield Gill Head, percentages of total land pollen. The stratigraphical units are identified and described in Table 1 and the FCS zones in the text (R, regeneration; I, interference; C, clearance).

Table 2 Preliminary results of radiocarbon determinations

		Radiocarbon age
Sample	No.	(yr b.p.)
I1 I2 I3 R4	HAR-4225 HAR-4226 HAR-4230 HAR-4229	$5,670 \pm 90$ $5,170 \pm 90$ $4,610 \pm 80$ $4,890 \pm 80$

We consider HAR-4230 to be anomalous and have ignored it in the

In the basal phase RO, Pinus sylvestris values are considerable, a feature common to early Flandrian II pollen spectra from the uplands of this region. Non-tree pollen values are also high, with Corylus, Salix and Gramineae prominent, as well as Pteridium spores. The subsequent pollen record implies that the open nature of the vegetation at this stage may be the legacy of an earlier episode of forest clearance, before the inception of peat formation at this site. From a peak at the base of the zone, non-tree pollen values decrease steadily as woodland regeneration continues. This trend towards more wooded conditions is abruptly reversed, however, during phase I1 (~5,670 yr b.p.), which marks the virtual demise of Finus sylvestris but sees distinct increases in Betula and Albus, with a sharp but temporary peak in the declining Corylus curve, echoed in the Pteridium spores. Fraxinus is recorded for the first time. Some ruderal pollen, including Melampyrum, Rumex, Cruciferae and Chenopodiaceae, show a first presence, or enhanced frequencies, as does a rather undifferentiated group like Rosaceae which may contain shrubs of open habitat. We interpret this interference phase as the result of forest necession associated with human activity4. Phase I1 is succeeded by a regeneration phase (R1) characterized, for example, by higher Quercus very high Betula, high Ulmus and Tilia levels, while Corylus and Fraxinus diminish, the latter to virtually nothing. With the exception of one horizon, Pteridium falls off in this phase as do ruderal pollen. Another I phase (I2, ~5,170 b.p.) follows at 55-58 cm, and then a short regeneration phase (R2) coinciding with a wood layer in the stratigraphy. The phases I2 and I3 are marked by especially high values for 'soot' and the fragments are sub-angular rather than rounded in shape, so that they may not have travelled far. Taxa like Corylus, Pteridium and heliophyte types Salix and Fraxinus all increase their frequencies in phase 12, while Melampyrum, Cirsium and Rumex are prominent among the ruderal herbs. Phase I3 (45-51 cm) records very abundant Pteridium and a large increase in the type and frequency of ruderal herbs, especially Melampyrum, Artemisia and Rumex, as well as frequencies for Calluna, which here exceed 100% of aggregate phase for the first time. This is presumably evidence for either a drier peat surface in the neighbourhood, or for the acidification of mineral soils, as it persists into regeneration phase R3. During the succeeding phase we detect the evidence of forest opening once again in the presence of Plantago lanceolata for the first time, along with high frequencies of Pteridium, Melampyrum and Betula: The pollen runs show that this phenomenon has a different character to the I phases and we have called it C1 (clearance 1). It is short lived and another R phase (R4) supervenes, to be interrupted by phase C2 near the op of the pollen diagram at a level where the sampling interval loes not permit its detailed discussion. The large Betula tree tump in the profile (~4,890 yr b.p.) corresponds with phase C1 ind the beginning of phase R4.

If the *Ulmus* decline marks the beginning of Neolithic sconomies in this region, then we should expect phases I1, I2 and I3 to result from Mesolithic man's activity. We infer that hey result from the burning of the woodland vegetation at or near the site as charcoal in the I phase is often quite large (15×9 mm pieces are found, for example). The 'soot' in the R phases probably came from burning in the region but far enough away to provide only background 'noise'. Even though doubts

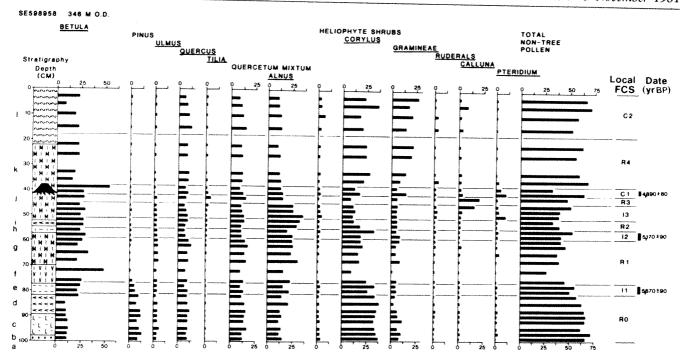


Fig. 2 Charcoal amounts at Bonfield Gill. The quantity of charcoal was estimated from disaggregated peat mixed with water which was spread over a gridded Petri dish. At  $\times 60$ , the percentage of each square ( $\Sigma = 100$ ) occupied by charcoal and carbonized material was estimated, J.B.I. estimates replicability to within ±3%. Note the correspondence with the I-phases identified from the pollen diagram (Fig. 1) and the lack of charcoal in R4: the post Ulmus decline phase on the pollen diagram.

have been expressed<sup>5</sup> on the interpretation of pollen diagrams in archaeological contexts, we believe that we have good evidence of repeated burning of the same site. The I-phase to I-phase interval is, however, longer than theory would predict<sup>2</sup> and more work is needed to see whether we are dealing with a real interval or whether we can improve the resolution of the technique: is R1 an aggregate phase, for example? Cessation of burning about the time of the Ulmus decline seems to have allowed tree growth and phases R1 and R2 could show smaller-scale evidence of the same feature. Even if the burning-recession sequence did not happen exactly here, it must have occurred in the near vicinity.

Finally, we caution that examination of many peat profiles from the North York Moors suggests that the Bonfield Gill site is unusual in its repetitive sequence, and that it is more common for wood to occur at the base of the blanket peat rather than above a sequence of peats and small wood fragments. While it may be that evidence of repeated burning, rather than the phenomenon itself, is patchy, it seems prudent to regard this practice as having had a limited spatial distribution. We emphasize, however, that the Bilsdale Moor area is said to have the highest density of Mesolithic flint sites in the country: this may be relevant circumstantial evidence. We suggest that here the burning seems to have stopped at the beginning of the Neolithic rather than later, as suggested for the southern Pennines; regional differences in occupance of the land by agricultural people could easily account for this point6.

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#### Successful biological control of the floating weed salvinia

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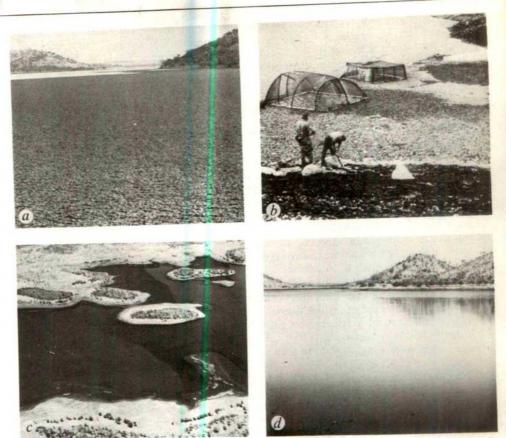
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The floating fern Salvinia molesta D. S. Mitchell (Salviniaceae) originated in southeastern Brazil<sup>1</sup>, but since about 1930, it has been introduced into various tropical and subtropical regions where it has become as great a menace as the water hyacinth<sup>2-4</sup>. Biological control of salvinia has clear economic and environmental advantages over other methods of control, but earlier attempts either failed or achieved inconclusive results<sup>5-7</sup>. We report here successful control of the largest salvinia infestation in Australia using the beetle Cyrtobagous singularis Hustache (Curculionidae) and suggest why this beetle has potential for controlling salvinia infestations elsewhere.

Salvinia was first recorded in Australia in 1952 and by 1976 it was present in many tropical and subtropical rivers and lakes and was more widespread than water hyacinth, which entered the country in the late 1800s8. Salvinia has achieved unacceptable population densities in Australia and elsewhere due to a combination of extremely high rate of growth, its dry weight doubling in 2.5 days in optimum conditions and an absence of suppressant parasites and pathogens8. By 1978 the largest infestation in Australia was on Lake Moondarra near Mt Isa, northern Queensland (20°36'S, 139°32'E), consisting of -50,000 tonnes fresh weight covering some 400 hectares (Fig. 1a). Attempts to control this infestation using herbicides

Fig. 1 S. molesta on Lake Moondarra in northern Queensland. a, In 1978. b, Weed damaged by C. singularis showed as dark patches on 20 January 1981, nearly 8 months after the beetles were released in and near the cages. c, The salvinia, forming a large mat to the left of the picture and smaller mats to the right of promontories, was under heavy attack by C. singularis and was uniformly dark brown on 17 May 1981. d, Most of the salvinia had been destroyed by 26 May 1981 (compare with a).



were abandoned in 1979 after expenditure of A\$160,000 (ref. 9).

The native range of *S. molesta* was unknown until 1978<sup>1</sup> and attempts at biological control until that time, in Africa, India, Sri Lanka and Fiji by the Commonwealth Institute of Biological Control, used insects found attacking the related *Salvinia auriculata* Aublet in northern South America and Trinidad<sup>5,7</sup>. In 1978, CSIRO Australia started a search for biological control agents in the newly discovered native range of *S. molesta* in southeastern Brazil. No new natural enemies were found, but local races of the three species of insect found by Bennett<sup>5</sup> to have potential for control were selected: *C. singularis*; the moth *Samea multiplicalis* (Guenée) (Pyralidae) which was released in northern Queensland in January 1981; and the grasshopper *Paulinia acuminata* (De Geer) (Pauliniidae) whose host specificity is being tested in quarantine in Australia.

Adult C. singularis are black, long-snouted weevils, about 2 mm long, which feed on salvinia buds. Eggs are laid among the submerged salvinia roots and larvae graze externally on roots before boring inside the rhizome.

Preliminary studies made in Brazil of *C. singularis* collected from *S. molesta* failed to detect any parasites or pathogens attacking the beetles, and to show whether the beetle could survive on plant species other than *S. molesta*. Eight hundred adults originally from Santa Catarina, Brazil, were then shipped to quarantine in Brisbane, Australia, where more detailed studies confirmed the absence of parasites and pathogens and the host specificity of *C. singularis*. Mass breeding was then started to obtain sufficient numbers for field releases.

On 3 June 1980 about 1,500 adults were released onto a salvinia-covered inlet on Lake Moondarra. About 500 beetles were introduced into each of two cages and the remaining 500 beetles were released immediately outside the cages. By late December, beetle activity had reduced salvinia cover by ~80% in the cages and caused discoloration of plants outside the cages. On 30 December fresh salvinia was added to the cages and the doors were left open.

Figure 1b shows the cages and the mouth of the inlet on 20 January 1981. The pale salvinia at the top left is healthy. A patch

of pale salvinia was blown around the square cage by strong winds the previous day and is nearly surrounded by darker salvinia damaged by *C. singularis*. Figure 2 shows the distributions of adult beetles and damage along a transect from the pale salvinia through the darker salvinia and further up the inlet. There was clearly a good correlation between the visual impression of damage from a distance and the proportions of leaves and buds damaged. It was also clear that beetles were moving away from heavily damaged salvinia, both onto newly available

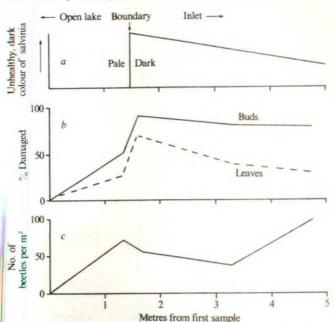


Fig. 2 Transect through the invasion front shown in Fig. 1b. a, Represents the sharp boundary between pale, healthy salvinia on the left and darker, unhealthy plants. b Shows the percentages of buds and leaves damaged by C singularis and c shows the numbers of adult C singularis per m<sup>2</sup> at five points along the transect.

undamaged plants and further up the inlet away from the population focus. No beetles or damage were found elsewhere on Lake Moondarra at this time.

On 20 January 1,500 more beetles were released onto salvinia about 300 m from the inlet and 10 large bags of beetle-infested salvinia were moved to the opposite side of the lake. A violent storm in February swept most of the remaining beetle-infested salvinia out of the inlet into the main body of the lake.

In late March salvinia throughout the lake started to turn brown and become waterlogged. On 18 April, all the salvinia was dark brown and samples taken at two sites 2 km apart contained respectively 60 and 80 adult C. singularis per m<sup>2</sup>, suggesting a total population of >100 million beetles. Population sizes of eggs, larvae and pupae were not estimated. Ninety-nine per cent of buds in both samples had been damaged. Damage was remarkably evenly distributed (Fig. 1c), suggesting an effective mode of beetle dispersal. A blacklight operated on the shore of the lake for 1 h from dusk was found to attract large numbers of strongly flying C. singularis.

By 26 May there was very little salvinia left (Fig. 1d). Three samples collected more than 1 km apart contained 100, 70 and 90 adult C. singularis per m<sup>2</sup>, which suggests a total population of about 6 million beetles. Ninety-nine per cent of the buds in each sample had been damaged.

Figure 3 presents estimates of the area and fresh weight of salvinia from August 1979 to 26 May 1981. The quantity of salvinia peaked in the autumn of each year until 1981. Drought starting early in 1978 reduced the autumn peak from 50,000 tonnes in 1978 to 19,000 tonnes in 1980 as the lake contracted in area to about 50% of maximum and salvinia was stranded. Substantial rain in January and February 1981 increased the area of the lake to ~80% of maximum and was expected to result in an autumn peak of 30,000 to 40,000 tonnes of salvinia. Some increase occurred to February 1981, after which C. singularis destroyed ~8,000 tonnes in 3 months and prevented significant regrowth. By August 1981 there was estimated to be <1 tonne of salvinia left. Despite the large quantity of salvinia killed and sunk, no algal blooms or other deleterious changes in water quality were detected (A. McCredie, personal communication). Based on analogous cases of biological control agents causing sudden collapses of dense populations of weeds<sup>10</sup>, we expect salvinia and C. singularis will be able to coexist on Lake Moondarra in a dynamic equilibrium at low population densities. However, both weed and control agent may become extinct on the lake if the remaining salvinia becomes stranded and dehydrated before the advent of the wet season in December 1981.

Why has C. singularis been so successful on Lake Moondarra, and has it the potential to control salvinia elsewhere? Attempts to establish the species on salvinia in Botswana and Zimbabwe in

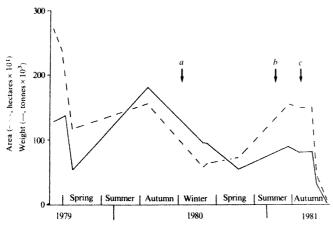


Fig. 3 Fluctuations in the area (---) and fresh weight (---)of salvinia on Lake Moondarra. a, First release of C. singularis; b, significant rainfall and second release + redistribution of beetleinfested plants; c, first appearance of widespread damage to salvinia. (Data courtesy of Mount Isa Mines Ltd.)

the early 1970s failed5, as did attempts to breed it in Fiji7. In these cases the beetles used were collected from S. auriculata in Trinidad, whereas the plant on which establishment was attempted was S. molesta. Although taxonomists recognize beetles collected from both species of plant as C. singularis, the Australian success may be due to our having obtained a race particularly adapted to S. molesta. If this is the case, we may have solved the intractable problems caused by S. molesta in Africa<sup>2,11</sup>, India<sup>2,4</sup>, Sri Lanka<sup>2</sup>, Indonesia<sup>2</sup>, Papua New Guinea<sup>3</sup>, New Zealand and Fiji<sup>7</sup>, and elsewhere in Australia.

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#### Enkephalin as a transmitter for presynaptic inhibition in sympathetic ganglia

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The endogenous opioid peptides, enkephalins, are known to be concentrated in discrete populations of central and peripheral neurones<sup>1-3</sup> and to inhibit synaptic transmission through a presynaptic mechanism reducing the release of transmitters<sup>4-6</sup>. These observations led to a hypothesis that enkephalin may serve as a transmitter for presynaptic inhibition at central and peripheral synapses<sup>4,5</sup>. To substantiate this hypothesis it is of crucial importance to demonstrate at specified synapses an inhibitory process that is evoked by stimulation of enkephalincontaining nerve fibres and is blocked by the opiate antagonist, naloxone. Mammalian sympathetic ganglia may provide a useful model for exploring this possibility, because enkephalin was shown, using immunohistochemistry<sup>3</sup>, to exist in certain nerve terminals in the ganglia and to inhibit cholinergic and noncholinergic transmission by a presynaptic mechanism<sup>5,7,8</sup>. We now report that in mammalian prevertebral ganglia, the conditioning stimulation given to preganglionic nerves produces a longlasting presynaptic inhibition of cholinergic transmission and that this inhibition is specifically abolished by naloxone. The results strongly suggest that enkephalin or closely related peptide(s) liberated from certain preganglionic fibres act as a transmitter for presynaptic inhibition, regulating the release of acetylcholine (ACh) in the sympathetic ganglia.

Experiments were carried out on the inferior mesenteric ganglia isolated from adult guinea pigs. The ganglion was placed in a bath and perfused with modified Tyrode solution at 36 °C. Intracellular recordings were made from ganglion cells as described previously<sup>5,8</sup>. Test stimuli were given to a small bundle of the lumbar splanchnic nerve through a suction electrode to evoke cholinergic fast excitatory postsynaptic potentials (e.p.s.ps). To the remaining preganglionic nerves, that is, the lumbar splanchnic and/or intermesenteric nerves,

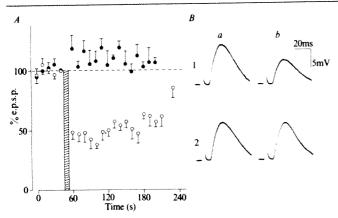


Fig. 1 Inhibitory effect of conditioning stimulation on cholinergic e.p.s.ps recorded from neurones in the inferior mesenteric ganglia, and antagonism by naloxone. Test e.p.s.ps were evoked by stimulating a small bundle of the lumbar splanchnic nerve at 1 Hz. Conditioning stimulation at 50 Hz for 8 s was given to the remaining lumbar splanchnic nerves during the period shown by the hatched column in A. A, eight successive e.p.s.ps were averaged every 10 s and recorded (see sample records in B). The amplitude of each averaged e.p.s.p. was expressed as a percentage of the control that was recorded immediately before the conditioning stimulation. Each point and vertical bar represent the mean ± s.e.m. obtained from three successive observations repeated at 6-min intervals on the same neurone. O, In control solution; and O, in the solution containing naloxone (3 µM). B, sample records of the averaged e.p.s.ps in another experiment. a, Test responses during the control periods; and b, conditioned responses obtained during the 15-20 s after the end of conditioning stimulation (50 Hz for 8 s). 1, In control solution; and 2, in the presence of naloxone  $(3 \mu M)$ .

conditioning stimulation was given with one or two separate suction electrodes.

Since the enkephalin-containing nerve terminals in the prevertebral sympathetic ganglia are thought to originate mainly from the preganglionic fibres  $^{3.9}$ , we sought to establish whether stimulation of preganglionic nerves produces an inhibitory effect on the cholinergic fast e.p.s.ps. After conditioning stimulation (50 Hz for 8 s), the amplitude of signal-averaged test e.p.s.ps was reduced to <50% of the averaged size of control e.p.s.ps, and the inhibition lasted for 2-4 min (Fig. 1). Such a prolonged inhibitory effect became obvious with the conditioning stimulation at 50 Hz for 2 s, and its magnitude depended on the number of conditioning stimuli. The maximal extent and the half-decay time of the inhibition produced by the preganglionic stimulation (50 Hz for 8 s) were  $70.5\pm4.7\%$  and  $56.4\pm5.8$  s respectively (mean  $\pm$ s.e.m. in 12 cells).

The inhibitory effect of the conditioning preganglionic stimulation on cholinergic e.p.s.ps was abolished by addition of naloxone (1-3 μM) (Fig. 1). Naloxone in the same concentration range almost completely blocked the inhibitory action of a potent enkephalin analogue, [D-Ala<sup>2</sup>]-Met-enkephalinamide  $(0.3-2 \mu M)$ , on cholinergic e.p.s.ps<sup>7</sup>. To test the specificity of the action of naloxone, we examined its effects on the inhibition produced by other substances. Noradrenaline (10-50 µM), dopamine (20-100  $\mu$ M) and  $\gamma$ -aminobutyric acid (20-50  $\mu$ M) depressed the cholinergic e.p.s.ps in the sympathetic ganglia<sup>10-12</sup>, but the inhibitory effects of these substances were not affected by naloxone (3  $\mu$ M). An  $\alpha$ -adrenergic blocker, phentolamine  $(1.5-3 \mu M)$ , on the other hand, abolished the effect of noradrenaline, but did not affect the neurally evoked inhibition of cholinergic fast e.p.s.ps. Furthermore, this inhibition remained unchanged in the presence of atropine (2 µM). Therefore, it is unlikely that catecholamine-containing interneurones are involved in this inhibitory process<sup>13</sup>

Further experiments were carried out to determine whether the mechanism of this inhibition is pre- or postsynaptic. The sensitivity of the postsynaptic membrane to ACh and the input resistance of ganglion cells were determined before and after conditioning stimulation. In the experiment shown in Fig. 2, both the sensitivity, to ACh and the membrane resistance of a ganglion cell increased slightly 10-60 s after the end of conditioning preganglionic stimulation (50 Hz for 8 s). In contrast, during the same period, the mean size of e.p.s.ps recorded from the same neurone was reduced to 15-60% of the control (Fig. 2a). The slight increase in the ACh-induced depolarization as well as in the membrane resistance may be due to the noncholinergic slow e.p.s.p. produced by preganglionic stimulation? These results can be explained by the hypothesis that the neurally evoked inhibition is caused mainly by a presynaptic mechanism reducing the amount of ACh release.

This hypothesis was further supported by quantal analysis of the e.p.s.ps during the control and conditioned periods (Fig. 3). On an assumption that the quantal release of ACh can be predicted by Poisson statistics<sup>14,15</sup>, the mean number of ACh quanta released by each test stimulus was estimated from the number of failures in a series of trials. During the period 10-170 s after conditioning stimulation (50 Hz for 12 s), the number of failures showed a ninefold increase over that observed during the control period (stippled columns in Fig. 3; see also Fig. 2a) and the mean quantum content of the e.p.s.ps was reduced to about 40% of the control. In contrast, the estimated size of the quantal unit did not change significantly after the conditioning stimulation (arrows in Fig. 3). Essentially the same results were obtained in eight other experiments, where the conditioning preganglionic stimulation (50 Hz for 8-12 s) reduced the mean quantum content to  $54.7 \pm 3.5\%$  of the control (mean ± s.e.m.) while the unit size of e.p.s.ps during the conditioned period was  $107.7 \pm 5.2\%$  of the control. The results, therefore, support the presynaptic nature of this neurally evoked inhibition. The effects of conditioning stimulation in increasing the number of failures and reducing the quantum content of e.p.s.ps were also reversed by naloxone (Fig. 3c). Naloxone alone, however, did not significantly affect the unit size or mean size of e.p.s.ps (Fig. 1B).

The present findings, together with other lines of evidence with regard to the action and the presence of enkephalin<sup>3,5</sup>,

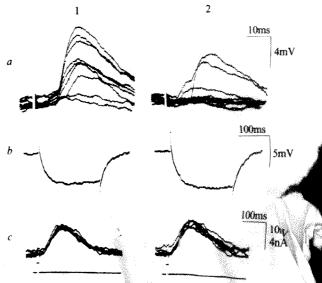


Fig. 2 Effects of conditioning stimulation on cholinergic e.p.s.ps. electrotonic potentials, and ACh-induced depolarizations recorded from a neurone in the inferior meseneric gangion and conditioning stimulation were given to the neurone with current pulses once every 2.5. a, Test Averaged electrotonic pontials produced by hyperpolarizing neurone at 1 Hz, eight rese were averaged. c, ACh-induced the ACh-containing elect (lower traces); five traces were and 2, response recorded 30 s after the end of conditioning

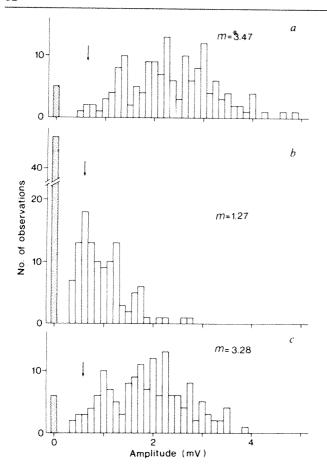


Fig. 3 Amplitude histograms of the control and conditioned cholinergic fast e.p.s.ps recorded from a neurone in the inferior mesenteric ganglion, and the effect of naloxone on the neurally evoked inhibition. Test e.p.s.ps were evoked at 1 Hz in a series of about 350 trials, and the conditioning stimulation (50 Hz for 12 s) was given at the middle of the series. Each histogram was compiled from the test responses in 160 trials. a, Amplitude distribution of test responses recorded during the control period before the conditioning stimulation. b, c, Amplitude distributions of conditioned test responses recorded during the periods 10-170 s after the end of the conditioning stimulation, a and b were obtained in control solution, and c in the solution containing naloxone (3  $\mu$ M). The quantum content, m, was estimated from the number of test stimuli (n = 160) and that of failures (stippled columns). The estimated unit size of the e.p.s.ps is shown by arrows.

pport the hypothesis that the neurally evoked presynaptic as sed from certain preganglionic fibres. Previous Collateral terminals of visceral afferent fibres to generate the prevented afference of the prevented noncholinergic slow e.p.s.p. in the prevertebral ganglion inhibits presupering MONICIONIUS BIC STORE Phalin inhibits presynaptically the Thus, the signal transfer and that Thus, the signal transfer processes incholinergic slow e.p.s. or only the classical cholinergic in the ganglia involve ow synaptic evens mediated probability of the ganglia involve ow synaptic evens mediated probability of the ganglia involve. the ganglia involve OW synaptic events mediated probably by sesion but also the OW synaptic events mediated probably by massion but also the duw synaptic enkehalin and substance P, peptider peic transmitters such as enkehalin and substance P, peptider peic transmitters such as enkehalin and substance P, period some anodulate the sympaetic tone regulation. peptitics and as compared and substance P, peptitics are modulate the sympaetic tone regulating the which serve to modulate the sympaetic tone regulating the which serve to modulate show that naloxone-record which serve to modulate the sympathic tone regulating the which serve to modulate the sympathic tone regulating the which serve to modulate the sympathic tone regulating the which serve to modulate the sympathic tone regulating the which serve to modulate the sympathic tone regulating the which serve to modulate the sympathic tone regulating the which serve to modulate the sympathic tone regulating the which serve to modulate the sympathic tone regulating the sympathic tone regulat inhibitory processes may exist in train areas of the central inhibitory processes may exist in the been suggestion. inhibitory processes may exist in the been suggested that nervous system 16,17. Furthermore nediated excitation nervous system by prinaptic mechanica. enkephalin inhibits the substance and excitation in the spinal trigeminal nucleus by prinaptic mechanism<sup>4</sup>. It is, spinal trigeminal nucleus by prinaptic mechanism<sup>4</sup>. spinal trigeminal nucleus by property incenanism. It is, therefore, likely that enkephalirs as a transmitter in presynaptic inhibition that controls release of ACh, substance P synaptic inhibition that controls remaining the central and second synaptic other transmittin central and second synaptic other transmitters. synaptic inhibition that controls in central and peripheral and possibly other transmitting synapses.

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#### Breakdown of cytoskeletal filaments selectively reduces Na and Ca spikes in cultured mammal neurones

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Cytoskeletal filaments, which contain microtubules <sup>1-4</sup> (~25 nm thick), microfilaments<sup>5,6</sup> (5 nm) and intermediate filaments<sup>7-9</sup> (neurofilaments, 10 nm), are considered essential for maintaining complex cellular shape and various cellular functions. Several studies have claimed that these filaments also maintain in some part physiological properties of excitable membranes<sup>10-14</sup>. However, the role of these filaments in maintenance of ionic channel activity is poorly understood 10,11. Here we report experiments in which we disrupted specific cytoskeletal filaments by applying chemicals to tissue-cultured nerve cells of adult guinea pigs<sup>15-17</sup>, and examined changes in membrane properties by conventional electrophysiological techniques. Selective breakdown of microfilaments by cytochalasin B<sup>5,6,18-21</sup> or its derivatives<sup>22-24</sup> caused selective reduction in the membrane capacity to carry Na ions 16 (as measured by  $\dot{V}_{\rm max}$  of pure Na spikes). The ability of the membrane to carry Ca ions1 and other physiological properties (resting membrane potential, input resistance and capacitance) were not affected significantly. By contrast, a breakdown of microtubules by colchicine  $^{1-4}$  or vinca alkaloids  $^{4,25}$  caused selective reduction of  $\dot{V}_{\rm max}$  for Ca spikes but not of that for Na spikes. These findings suggest that Na channels in the plasma membrane may have a molecular interaction with microfilaments, but are independent of microtubules. Conversely, Ca channels may be related to microtubules or to neurites, but seem to be independent of microfilaments.

The techniques used in tissue culture of nerve cells from adult guinea pigs have been reported elsewhere 15-17. Dorsal root ganglia were incubated with collagenase in L-15 medium, and nerve cells isolated from the ganglia by vibration. The nerve cells were grown in collagen-coated plastic dishes on growth medium (75% Eagle's medium, 15% fetal calf serum, 9% chick embryo extract and 1% penicillin-streptomycin solution) in air containing 5% CO2 at 37°C.

After 2 days of culture, cytochalasin B and colichicine, prepared as described below, were added to the tissue culture dishes (1.5 ml growth medium per dish) using a microsyringe. At this stage of culture, nerve cells were starting to extend their neurites 15,17 (Fig. 1a). Cytochalasin B (1 mg) was first dissolved in dimethyl sulphoxide (DMSO, 1 ml), then diluted with L-15 medium to give a final concentration in stock solution of

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Table 1 Changes in resting membrane properties and Na spike component of cultured nerve cells after exposure to cytochalasin B and colchicine

		Resting membrane potential (mV)	Input resistance $(M\Omega)$	Input capacitance (pF)	$\dot{V}_{\rm max}$ of Na spikes $({\rm V~s}^{-1})$	Ratio
Cytochalasin B Control Cytochalasin B	(4 μg ml <sup>-1</sup> , 2 days) (DMSO) (6 μg ml <sup>-1</sup> , 2 days) (L-15)	$-50\pm11 \ (n=19)$ $-51\pm9 \ (15)$ $-55\pm10 \ (15)$ $-54\pm15 \ (7)$	$35 \pm 13$ $34 \pm 10$ $42 \pm 18$ $38 \pm 12$	$45 \pm 15$ $41 \pm 16$ $56 \pm 18$ $50 \pm 15$	$76 \pm 22 \ (n = 16)$ $113 \pm 31 \ (15)$ $89 \pm 18 \ (13)$ $128 \pm 18 \ (6)$	0.67* 0.70*
Control (L-15) Cytochalasin B (12 $\mu$ g ml <sup>-1</sup> , 1 day) Control (L-15)	$-48 \pm 6 (7)$ $-50 \pm 6 (7)$	$36 \pm 10$ $33 \pm 9$	$49 \pm 15$ $52 \pm 19$	$34 \pm 11 (6)$ $121 \pm 25 (5)$	0.28†	
Colchicine Control	(8 μg ml <sup>-1</sup> , 2 days) (L-15)	$-50 \pm 8 (20)$ $-52 \pm 9 (17)$	$35 \pm 11$ $32 \pm 14$	$46 \pm 16$ $53 \pm 18$	100 ± 34 (17) 118 ± 55 (15) 108 ± 36 (16)	0.85
Colchicine Control	(16 μg ml <sup>-1</sup> , 2 days) (Lumicolchicine)	$-45\pm7$ (18) $-47\pm8$ (21) $-47\pm7$ (15)	$30 \pm 5$ $32 \pm 19$ $29 \pm 10$	$43 \pm 17$ $45 \pm 18$ $77 \pm 30$	$108 \pm 30 (16)$ $110 \pm 32 (14)$ $154 \pm 32 (11)$	0.98
Colchicine Control	$(20 \mu g \text{ ml}^{-1}, 1 \text{ day})$ (L-15)	$-54 \pm 7 (15)$ $-52 \pm 8 (12)$	$31 \pm 12$	$68 \pm 28$	161 ± 43 (11)	0.96

All values are mean  $\pm$  s.d.; numbers of neurones examined are indicated in parentheses. The nerve cells were immersed in a  $\text{Co}^{2+}$ - and  $\text{TEA}^+$ -containing medium (see Fig. 2 legend), in which the nerve cells generated pure Na spikes. Input resistance and capacitance were obtained by intracellular passage of a small hyperpolarizing pulse current (up to 0.3 nA for 16 ms), assuming that the membrane was a single resistance-capacitance circuit. Pure Na spikes were elicited, as seen in Fig. 2;  $\dot{V}_{\text{max}}$  of the spikes were obtained by electronic differentiation. Before passage of pulse current the membrane potential of the penetrated nerve cells was held at  $\sim -100 \, \text{mV}$ , where the current-voltage relationship is approximately linear and the membrane generates a full-sized Na spike. Note that no change was induced in resting membrane properties of the nerve cells after exposure to cytochalasin B and colchicine.

P < 0.01; † P < 0.001 (Student's t-test).

100 μg ml $^{-1}$ . Dihydrocytochalasin B $^{23,24}$  and cytochalasin D $^{22}$  were prepared in the same way. Control solutions consisted of either L-15 medium alone or L-15 medium containing 10% DMSO. Colchicine, vinblastine sulphate, vincristine sulphate and colcemide were stored as solutions (2 μM) in L-15 medium. Control solutions for these alkaloids were either L-15 medium alone or L-15 medium containing 800 μg ml $^{-1}$  (2 μM) lumicolchicine (prepared by UV illumination of colchicine dissolved in 99% ethanol $^{25}$ ).

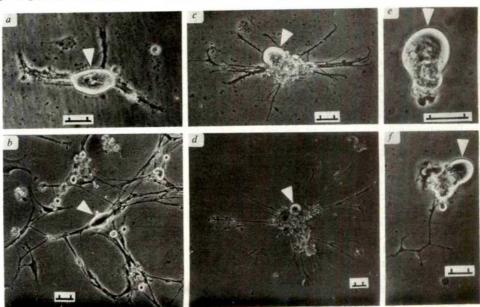
Cultured nerve cells were penetrated by a glass micro-electrode  $(20\text{--}30~\text{M}\Omega)$  filled with 3 M KCl. To elicit spikes and measure membrane resistance and capacitance, d.c. and pulse currents (up to 5 nA) were passed intracellularly through the microelectrode. The intracellular potential was determined by a Wheatstone bridge balance. Input resistance and capacitance were measured using a small pulse current while holding the membrane potential at  $\sim -100~\text{mV}$ ; the nerve cell was assumed to be a single RC circuit<sup>16</sup>. Na and Ca spikes were elicited using depolarizing pulses. Pure Na spikes were elicited in nerve cells bathed in a Co<sup>2+</sup>-containing solution (see ref. 16 and Fig. 2 legend); Co<sup>2+</sup> was added to inhibit Ca spikes<sup>26</sup>, which otherwise are superimposed on the Na spikes. Pure Ca spikes were elicited in nerve cells bathed in a Na-free tetraethylammonium(TEA)-containing solution (see ref. 15 and Fig. 2 legend). Before spike

initiation, the membrane potential of the penetrated nerve cell was hyperpolarized to a potential more negative than  $-100~\rm mV$ . This continuous hyperpolarization eliminated inactivation of Na and Ca channels which occurs at a small resting membrane potential  $^{15-17}$  (Table 1). The  $\dot{V}_{\rm max}$  of a full-sized spike elicited during the hyperpolarization was obtained by electronic differentiation; this  $\dot{V}_{\rm max}$  was considered proportional to the largest amplitude of inward Na or Ca currents in the membrane  $^{27}$ , and was compared for nerve cells grown in different conditions.

Cytochalasin B caused several distinct morphological changes in nerve cells (Fig. 1c, d). (1) Growth of neurites was reduced at a low dose ( $4 \mu g \, \text{ml}^{-1}$ ) and suppressed at the higher dose of  $12 \, \mu g \, \text{ml}^{-1}$ : nerve cells tended to have fine neurites that were relatively short. (2) Neuronal somata became spherical; and (3) within a few days of exposure, the nerve cell population was reduced (2/3 to 1/2) due to loss of nerve cells from the monolayer. (4) Growth of fibroblasts and Schwann cells was inhibited. These changes can be regarded as selective breakdown of microfilaments by cytochalasin  $B^{5,6,18-21}$ . DMSO alone, added as a control, caused no significant morphological or population change in both neuronal and glial cells (results not shown).

Intracellular recordings of nerve cells exposed to cytochalasin

Fig. 1 Changes in morphology of cultured nerve cells after exposure to cytochalasin B or colchicine. a. Nerve cell which, after 2 days in tissue culture, started to extend neurites from the somata (triangle). b. Control nerve cell (triangle) after 4 days of culture. c, d, Neurones after exposure to cytochalasin B. These cells were maintained in culture for 4 days, and exposed to cytochalasin B at a concentration of 12 µg ml<sup>-1</sup> or 4 µg ml<sup>-1</sup> (d) during days 2 to 4. Note the sparse neurite growth compared to control cells (b). e, f, Neurones exposed to colchicine (16 µg ml<sup>-1</sup>) during days 2 to 4 of tissue culture. Most nerve cells became spherical (e). Some cells had fine neurites of a very short length (f). Calibration bars: 20 μm.



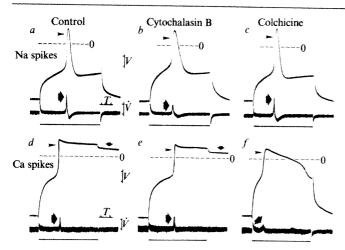


Fig. 2 Na and Ca spikes elicited in cultured nerve cells exposed to cytochalasin B or colchicine. Na spikes (wedges in a-c) and Ca spikes (wedges in d-f), elicited in normal cells (a, d), cytochalasin B-treated cells  $(4 \mu g ml^{-1}; b, e)$  and colchicine-treated cells  $(16 \mu g ml^{-1}; c, f)$ . All nerve cells were maintained in vitro for 4 ; c, f). All nerve cells were maintained in vitro for 4 days, and were exposed to the chemicals during days 2 to 4 of tissue culture. Na spikes were elicited in nerve cells in medium containing 141 mM NaCl, 1 mM CoCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 10 mM TEA-Cl, 5.5 mM KCl, 5.5 mM glucose and 2 mM Na-HEPES at pH 7.4. Ca spikes were elicited in Na-free medium containing 80 mM TEA-Cl, 63 mM Tris-HCl, 10 mM CaCl<sub>2</sub>, 5.5 mM KCl and 5.5 mM glucose, pH 7.4. Downward arrows in d and e show that overshoots of Ca spikes were still present even after cessation of depolarizing pulse-current passage. However, Ca spikes in colchicine-treated nerve cells were of short duration (f). Lower traces of a-f were obtained by electronic differentiation of the upper intracellular records.  $V_{\text{max}}$  of the spikes (arrows) were considered to be proportional to transient inward Na or Ca currents associated with the respective spikes. Bars underneath indicate the duration of depolarizing pulse currents passed intracellularly. Calibrations: T, 2 ms for a-c and 20 ms for d-f; V, 20 mV for a-f;  $\dot{V}$ , 80 V s<sup>-1</sup> for a-c and  $10 \text{ V s}^{-1}$  for d-f. Further details are given in the text.

B for 1-2 days revealed resting membrane properties (resting potential, input resistance and capacitance, and I-V relationship) that were very similar to those of normal nerve cells (Table 1). The nerve cells were also capable of generating both Na and Ca spikes (Fig. 2b, e). The most remarkable effect of cytochalasin B exposure was a selective reduction in  $\dot{V}_{\rm max}$  of the Na spikes (arrow in the lower trace of Fig. 2b, and Table 1). This reduction, which was small at 6 h (85% of normal value, P< 0.2), became apparent (P < 0.01) in 2 days (Table 1). A higher dose of cytochalasin B induced a larger reduction in a shorter period (Table 1). However, this was associated with a great loss of cells. This reduction in the  $V_{\text{max}}$  of the Na spikes disappeared 2 days after removal of cytochalasin B from the growth medium (data not shown). Similar specific reduction in the Na spikes was induced by dihydrocytochalasin B and cytochalasin D (results not shown) which also cause breakdown of microfilaments<sup>22-24</sup> By contrast, there was no change detected in Ca spikes (Fig. 2e and Table 1), even when dose and time of exposure to cytochalasin B were increased. These results indicate that the mechanisms required to generate Ca spikes are independent of microfilaments.

Exposure of the cultured nerve cell to colchicine  $(16 \ \mu g \ ml^{-1})$  for 2 days caused retrogression of neurites (Fig. 2c, f), and produced spherical somata and a reduction in nerve cell population. Colchicine inhibited growth of fibroblasts and Schwann cells on the dishes, which can be explained by a breakdown of microtubules by colchicine<sup>1,3,4</sup>. Lumicolchicine  $(128 \ \mu g \ ml^{-1})$ , added as a control, did not change neuronal and glial morphology (results not shown). The most remarkable changes in membrane properties effected by colchicine were a small  $V_{\rm max}$  and a short duration of Ca spikes (Fig. 2f and Table 2). These changes in the Ca spikes appeared within 1–2 days of colchicine application, but disappeared 3 days after its removal (not

shown). A higher dose of colchicine or a longer period of exposure resulted in only a slightly smaller  $\dot{V}_{\rm max}$  (Table 2). By contrast, no statistically significant change (P < 0.2) was observed for Na spikes (Fig. 2c, Table 1), resting potential, input resistance and capacitance (Table 1). A similar specific reduction in  $\dot{V}_{\rm max}$  of the Ca spikes was induced by other vinca alkaloids (vinblastine, vincristine and colcemide; data not shown)—these also disrupt microtubules  $^{1-4.25}$ .

These experiments demonstrate that, even though cytochalasin B and colchicine induce drastic changes in cell morphology, many nerve cells exhibit healthy electrophysiological properties. In particular, the lack of significant change in input resistance and capacitance (first order approximation) indicates that the basic structure and function of the plasma membrane seem to be maintained. Assuming that capacitance of unit area of the plasma membrane remained unchanged, the small  $\dot{V}_{\rm max}$  of the Na spikes induced by cytochalasin B is considered to be due to a reduction in inward Na currents<sup>27</sup> which may be explained further as a reduction in Na channel density in the membrane or as a conformational change in Na channels. Reduction in electromotiveforce of Na current does not seem to be a main cause because Na spikes in the cytochalasin B-treated nerve cells showed a large overshoot or, for a few cells, only a small reduction in spike amplitude (threshold to peak) of ~10 mV. It is highly unlikely that K currents were increased by cytochalasin B because the K conductance should, in part, affect Ca spikes.

The selective reduction in Na spikes by cytochalasin B suggests an interaction between microfilaments and Na channel molecules<sup>10</sup>. However, this interaction may not always be simple, as at least 24 h are required to induce an apparent change in the Na spikes. Possible interactions are: (1) microfilaments may constitute major anchoring filaments of Na channel molecules in the plasma membrane; (2) microfilaments may be involved in transportation of Na channel molecules into the plasma membrane; (3) they may contribute to synthesis of Na channels in nerve cells. By contrast, the fact that colchicine had little effect on Na spikes indicates that the Na channels in the membrane may be independent of microtubules<sup>11</sup>.

The reduced Ca spike caused by colchicine treatment of nerve cells suggests an interaction between microtubules and Ca channel molecules: that is, microtubules may contribute to maintaining Ca channels in the plasma membrane, for example, as an anchoring cytoskeletal filament. However, some observations do not conform to this idea. First, electron-microscopical observations have generally failed to demonstrate microtubules at presynaptic nerve terminals, which are presumed to be rich<sup>26,28</sup> in Ca channels. In a few studies where microtubules are found in nerve terminals, they seem to be

Table 2 Changes in Ca spike component of cultured nerve cells exposed to cytochalasin B and colchicine

		$\dot{V}_{\max}$ of Ca spikes $(\mathbf{V}  \mathbf{s}^{-1})$	Ratio
Cytochalasin B Control Cytochalasin B Control	(6 μg ml <sup>-1</sup> , 2 days) (DMSO) (10 μg ml <sup>-1</sup> , 3 days) (L-15)	$4.8 \pm 2.5 \ (n = 12)$ $4.9 \pm 1.8 \ (14)$ $8.0 \pm 2.1 \ (11)$ $8.7 \pm 2.8 \ (8)$	0.98 0.92
Colchicine Control Colchicine Control Colchicine Control	(16 µg ml <sup>-1</sup> , 1 day) (L-15) (32 µg ml <sup>-1</sup> , 2 days) (Lumicolchicine) (4 µg ml <sup>-1</sup> , 3 days) (L-15)	$2.7 \pm 1.6$ (9) $4.6 \pm 1.2$ (12) $4.6 \pm 2.1$ (11) $9.1 \pm 3.3$ (16) $4.1 \pm 1.8$ (12) $8.7 \pm 2.8$ (8)	0.59* 0.51† 0.47†

Ca spikes were elicited in nerve cells immersed in a Na-free, TEA<sup>+</sup>-rich medium, as seen in Fig. 2. All values are mean ± s.d.; numbers of sampled nerve cells are indicated in parentheses. Note that there was no significant change in Ca spikes for nerve cells exposed to cytochalasin B. For further details see text.

<sup>\*</sup> P < 0.02; † P < 0.01 (Student's *t*-test).

related to synaptic vesicles but not to the plasma membrane<sup>29</sup> Second, microtubules are easily depolymerized by Ca ions<sup>4,30</sup> which pass through the Ca channels. Thus, we cannot exclude the possibility that the change in the Ca spikes may be due to wide changes in cellular structure, such as changes in cell shape. Indeed, Ca spikes become large during growth of neurites in tissue culture 15, and are considered to form major action potentials in dendrites of cerebellar Purkinje cells31. Thus, the small Ca spikes may be due to a loss of neurites caused by microtubule breakdown after colchicine treatment of the nerve cells

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#### Immunochemical and immunocytochemical localization of S-100 antigen in normal human skin

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The S-100 antigen1 is generally considered to be unique to the nervous system, where it is found primarily in the cytoplasm and nucleus of glial cells, both in soluble and bound form<sup>2,3</sup>. It belongs to the family of acidic Ca2+-binding proteins4. In phylogenesis, S-100 conserves a close immunological relationship between different species, and during ontogenesis the pattern of its accumulation parallels the functional maturation of the nervous system<sup>2,3</sup>, although its biological role remains to be clarified. Recently, S-100 has been found in cells of non-nervous organs (interstitial cells of the pineal gland5, stellate cells of the adenohypophysis<sup>6,7</sup> and satellite cells of the adrenal medulla<sup>8</sup>), and in cultured malignant human melanomas?. We report here the presence of S-100 in normal human skin, where the antigen seems to be located specifically in melanocytes and in cells with morphological features of Langerhans cells.

Table 1 S-100 protein concentration in normal human skin and malignant human melanoma

	μg S-100 per mg soluble
Tissue	protein
Normal skin Malignant melanoma	0.03 1.90

Normal human skin (0.5 g per experiment) and malignant human melanoma (1.0 g per experiment), tested by histopathology, were obtained from the neck and leg during surgery (three specimens each of normal human skin and human melanoma). The tissues were homogenized in a glass homogenizer in 10 mM Tris-HCl, pH 7.4 (1:4 w/v), containing 0.1 mM phenylmethylsulphonylfluoride (Sigma) to minimize proteolytic degradation<sup>21</sup>. The homogenates were centrifuged at 105,000g for 60 min at 4 °C and the supernatants analysed for total protein by the method of Lowry et al.<sup>22</sup>, and for S-100 content by microcomplement fixation<sup>10</sup> using as a standard ox S-100 purified as described by Moore1. Samples with complement alone and with both complement and antiserum were assayed to test the anticomplementary activity of tissue extracts and antiserum. S-100 values are expressed in terms of ox S-100 equivalents. All measurements were performed in duplicate; all values were corrected for anticomplementary activity, when present. The antiserum was diluted 1:500 before the assay. Maximal s.e.m. was  $\pm 5\%$  (n = 3). The bound fraction was not evaluated.

Levels of S-100 were measured by microcomplement fixation assay10, both in normal skin and, for comparison, in human melanomas, using a specific antibody raised against ox S-100 and characterized as described elsewhere<sup>11</sup>. Cross-reactivity between ox and human S-100 protein has been observed<sup>11,12</sup>. The complete complement fixation curve for extracts of normal human skin and a standard curve of S-100 protein are shown in Fig. 1a, b. The amounts of S-100 were measured in samples containing 15 or 25 µg total protein (~100-165 µg protein ml-1); no appreciable anticomplementary activity was observed at these concentrations. The S-100 antigen was detected in measurable amounts in both normal skin and melanoma, with an ~80 times higher concentration in the latter than in the former (Table 1). The concentration of S-100 in the skin seemed to be ~100 times lower than that reported for the human brain9.

The cellular and subcellular localization of S-100 in normal human skin was also investigated by electron microscopy using unlabelled antibody PAP method13 with the same

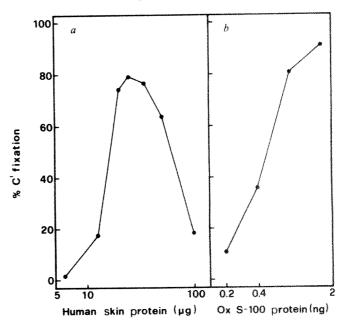


Fig. 1 Representative complement (C') fixation curves of S-100 from normal human skin (a), and of purified ox S-100 (b). Normal skin extract and purified S-100 were prepared, and quantitative complement fixation performed as described in Table 1 legend. All values were corrected for anticomplementary activity, when present.

antiserum. In the epidermis, S-100 reaction product was detected in the perikarya and in the cytoplasmic processes of both melanocytes and Langerhans cells (Fig. 2a, b). No reaction product was present in the keratinocytes of any epidermal layer (Fig. 2a). Merkel cells were not observed in our preparations. In the dermis, S-100 reaction product was detected not only in typical Langerhans cells (which appeared as elongated cells with indented nuclei and slender processes lying close to the other cell types, exhibiting the distinctive cytoplasmic granules; Fig. 2c), but also in cells with ultrastructural features comparable with Langerhans cells but lacking the characteristic granules (Fig. 2d). It is possible that these are Langerhans cells having granules that are not detectable in the plane of the section, or 'histiocytes' or 'dermal macrophages' having the latent capacity to become manifest as dermal Langerhans cells by production of the characteristic granules14. Fibroblasts and collagen fibres, lymphocytes, vascular endothelial cells and pericytes had no reaction product. Melanocytes and nerve fibres were not detected in the dermis.

The subcellular distribution of the antigen was the same in all the S-100-immunoreactive cells. Immunoreactivity was widely distributed in the cytoplasmic matrix but absent from the cisternae of the endoplasmic reticulum and the Golgi apparatus, as well as from the plasma membranes and mitochondria (Fig. 2a-d). Melanosomes and Langerhans granules also appeared free of reaction product (Fig. 2a-c). In the nucleus, immunoreactivity was found in the nucleoplasm but not in the nucleolus or the nuclear envelope (Fig. 2a, b, d). Control experiments carried out using preimmune rabbit serum or anti-S-100 antiserum absorbed with the antigen did not show any

reaction product (Fig. 3a, b). Thus the distribution of the antigen in immunoreactive cells of the skin seems similar to that reported for S-100-immunoreactive cells of nervous  $^{15.16}$  and non-nervous organs  $^{5-8}$ .

Our findings agree with recent observations on the presence of S-100 in malignant human melanomas<sup>9</sup>. In addition, they indicate that definite cutaneous cell types normally contain significant amounts of the antigen, but at a much lower concentration than in malignant tissue. No detectable amounts of antigen in normal human skin were reported by Gaynor et al.<sup>9</sup> as tested by microcomplement fixation assay; this discrepancy could be accounted for by use of different titres of antisera. The presence of S-100 in normal melanocytes rules out the possibility of the protein being regarded as a marker unique to malignant melanocytes. In this respect, detailed studies will be needed to establish any possible relationship between levels of S-100 and the degree of malignancy of melanomas, as reported for human tumours of glial origin<sup>17</sup>.

The presence of S-100 in melanocytes agrees with the neuro-ectodermal origin of this cell type <sup>18,19</sup>. On the other hand, the observation that Langerhans cells contain S-100 is intriguing, as these are generally believed to be mesodermal in origin <sup>18,20</sup> and data on the cellular localization of S-100 indicate that the protein is present in cells commonly considered to be of neuro-ectodermal origin <sup>2,3</sup>. It is also possible that Langerhans cells take up S-100 passively, although the distribution of the antigen both in the cytoplasm and the nucleus, similar to that observed in other S-100-immunoreactive cells <sup>5-8,15,16</sup>, seems to oppose this view.

Our present data, together with the recent findings of S-100

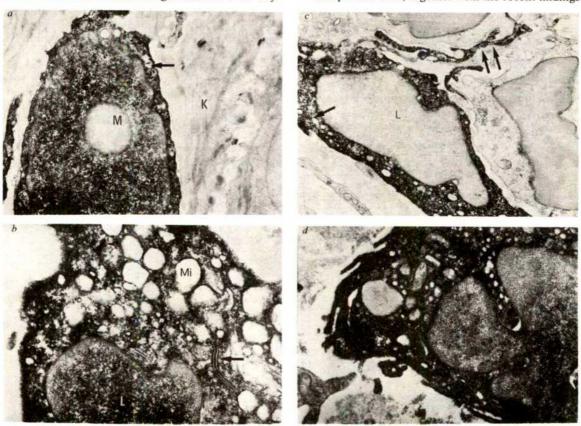
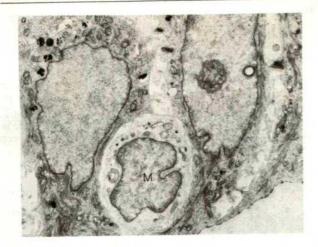


Fig. 2 Sections of normal human skin reacted with anti-S-100 antiserum. Normal human skin obtained from the neck or leg regions during surgery on three patients was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 3 h, washed in phosphate-buffered saline (PBS) overnight and then sectioned on a Sorvall TC-2 chopper. The sectioned slices were treated with anti-S-100 antiserum diluted in PBS at 1:500, using the unlabelled antibody PAP method as described elsewhere<sup>8</sup>. a, The melanocyte (M) is labelled in the nucleoplasm as well as in the cytoplasmic matrix. No reaction product is present in the nucleoplas and in melanosomes (arrow). The keratinocytes (K) do not show any reaction product. b, The epidermal Langerhans cell (L) shows immunoreactivity in the nucleoplasm as well as in the cytoplasmic matrix. The mitochondria (Mi) and the typical cytoplasmic organelles (arrow) are free of reaction product. c, The dermal Langerhans cell (L) with the distinctive organelles (arrow) shows reaction product in the cytoplasmic matrix. No reaction product is detectable in the nucleus, possibly due to insufficient penetration of antibodies during the immunocytochemical procedure, as also observed elsewhere <sup>7,8,16</sup>. Labelled Langerhans cytoplasmic processes (double arrow) are closely related to other cell types which seem to lack immunoreactivity. d, The dermal Langerhans-like cell shows S-100 immunoreactivity in the nucleoplasm as well as in the cytoplasmic matrix. a, ×11,400; b, ×23,000; c, ×11,400; d, ×18,300.



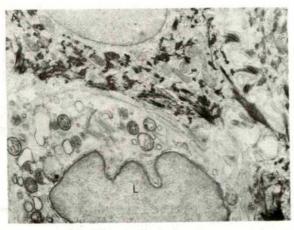


Fig. 3 Control sections of normal human skin treated as described in Fig. 2 legend but reacted with anti-S-100 antiserum absorbed with S-100 antigen. Neither the melanocyte (M) nor the epidermal Langerhans cell (L) show any reaction product. The same results were obtained when sections were treated with normal preimmune rabbit serum. a, ×9,400; b, ×14,300.

antigen in non-nervous organs in normal conditions5-8, suggest that S-100 should no longer be considered strictly as a protein specific to the nervous system. Further studies on the general tissue distribution of S-100 will be needed to clarify whether the protein is confined to cell types of common embryonic origin.

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#### Isoleucine-requiring Nicotiana plant deficient in threonine deaminase

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Well characterized auxotrophic mutants would greatly assist the genetic manipulation of flowering plants, but such mutants are rare1-3. Moreover, auxotrophic mutants isolated at the plant level4-8 and some of those isolated in cell cultures9 are leaky. The isolation of the first non-leaky auxotrophic mutant, requiring a source of nitrogen in reduced form, was possible by direct selection for chlorate resistance of this rare mutant type 10. A different approach, the individual testing of colonies grown from mutagenized haploid cells of Datura 11 and Hyoscyamus 12, has resulted in cell lines with absolute nutritional requirement. We report here the isolation of an isoleucine-requiring line from haploid cell cultures of Nicotiana plumbaginifolia and the regeneration of diploid plants. These plants have no detectable activity of L-threonine deaminase (EC 4.2.1.16), the first enzyme in isoleucine biosynthesis.

Haploid protoplasts were prepared from leaves of N. plumbaginifolia plants, mutagenized by irradiation and grown to small colonies in complete medium. Calli derived from nonirradiated protoplasts were not studied. Two out of 11,049 colonies tested after mutagenesis were unable to grow on minimal medium (Table 1); they were later identified as a uracil (URA401) and an isoleucine (ILE401)-requiring line by growing them on media supplemented with various amino acids, vitamins and nucleic acid bases following the test system of Holliday<sup>13</sup>. Normal growth of URA401 and ILE401 (Fig. 1) could be restored by feeding 100 mg l-1 uracil and 200 mg l-1 isoleucine, respectively. Characterization of the URA401 line will be reported elsewhere.

Plants could be regenerated only from the ILE401 line. The regenerates were diploid (Fig. 2), although selection was carried out using haploid cells. Diploidy can be explained by spontaneous polyploidization known to occur frequently in cultured plant cells<sup>14</sup>. The plants, and the calli initiated from them, have an absolute requirement for isoleucine.

Table 1 The frequency of auxotrophic clones

		No. (f	requency) of
Dose $(J kg^{-1})$	% Survival	clones	auxotrophs
13	57	3,727	0
16	23	3,705	0
19	15	2,377	0
23	11	1,240	$2(1.6\times10^{-3})$

Haploid N. plumbaginifolia protoplasts were isolated from sterile plants<sup>21</sup>, irradiated with 60Co γ rays<sup>22</sup> and cultured in K3 medium<sup>2</sup> supplemented with 0.1% casein hydrolysate and 0.05% yeast extract. The colonies were then individually transferred onto RM medium<sup>24</sup> containing 0.1 mg l<sup>-1</sup> 1-naphthaleneacetic acid and 1.0 mg l<sup>-1</sup> benzyladenine (RMOP medium) and the same supplements as before. Calli (5-10 mm in diameter) were then halved and subcultured onto minimal (casein hydrolysate and yeast extract omitted) and supplemented RMOP medium. Auxotrophs were identified by their inability to grow on minimal medium. Dese rate was 0.042 J per kg per s. % Survival = of non-irradiated control. Plating efficiency in cultures of non-irradiated protoplasts was ~70%.

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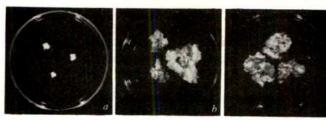


Fig. 1 Nutritional requirement of the ILE401 line. a, Minimal RMOP (see Table 1 legend) medium; b, minimal medium with 200 mg l<sup>-1</sup> L-isoleucine; c, minimal medium with 100 mg l<sup>-1</sup>  $\alpha$ aminobutyric acid. Cultures were incubated for 3 weeks in the light (2,000 lx, 16 h).



Fig. 2 Wild-type diploid N. plumbaginifolia (a) and ILE401 (b) plants. Metaphase chromosomes in root tips of an ILE401 auxotrophic plant are also shown (c).

Except for the first and last steps of their biosynthetic pathway, isoleucine and valine are synthesized by a common set of enzymes in microorganisms15 and in flowering plants16. As isoleucine alone is sufficient to restore normal growth of the ILE401 line, it may be defective in either threonine deaminase or transaminase B (Fig. 3). There was no detectable threonine deaminase activity in crude extracts of the ILE401 line, in contrast to the wild type, in which the specific activity was  $1.0 \times 10^{-3} \,\mu$ mol per min per mg protein, a value comparable with those found in other plant species 17,18. Transaminase B activity, however, was not affected in the mutant. Specific activities in the mutant and wild type were  $5.2 \times 10^{-4}$  and  $3.9\times 10^{-4}~\mu mol$  per min per mg protein, respectively. (Details of the assays of both enzymes are given in Fig. 3 legend.) The isoleucine requirement of the ILE401 line is therefore due to the absence of threonine deaminase activity. Normal functioning of the rest of the pathway was shown in growth tests in which isoleucine could be substituted with  $\alpha$ -aminobutyric acid (Fig. 1), which is converted in vivo to  $\alpha$ -ketobutyric acid<sup>19</sup>, the product of threonine deaminase. To avoid complications due to feedback inhibition by excess isoleucine20, α-aminobutyric acid was used in the test, rather than  $\alpha$ -ketobutyric acid, to provide a physiological concentration of  $\alpha$ -ketobutyric acid (thus indirectly that of isoleucine).

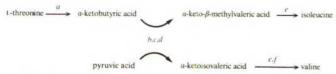


Fig. 3 The isoleucine-valine pathway. a, Threonine deaminase; b, acetohydroxy acid synthase; c, acetohydroxy acid isomeroreductase; d, dihydroxy acid dehydrase; e, transaminase B; f, transaminase C. Extracts for the enzyme assays were prepared by grinding 1 g leaf tissue in 3 ml buffer (50 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM 2-mercaptoethanol (2-ME), 2 mM EDTA, pH 8.0) and centrifuging the homogenate at 15,000g for 10 min. Threonine deaminase activity was measured at 37 °C in the presence of 62.5 mM KH<sub>2</sub>PO<sub>4</sub>, 40 mM L-threonine, 5 mM 2-ME, 0.5 mM EDTA, pH 8.0.  $\alpha$ -ketobutyric acid formed was determined by the direct method of Friedemann and Haugen<sup>25</sup>. Transaminase B activity was measured at 37 °C in the presence of 30 mM α-ketoglutaric acid, 25 mM L-isoleucine, 12.5 mM KH2PO4, 5 mM 2-ME, 0.5 mM EDTA and 0.1 mM pyridoxal phosphate, pH 8.0, using the reverse reaction. α-keto-β-methylvaleric acid formed was determined by the indirect method of Friedemann and Haugen2

The isoleucine requirement of the ILE401 line may be a result of a mutation or of epigenetic changes3. The low frequency of this phenotype, stability in culture for more than a year and expression at the plant level support the former3. Preliminary data on fusion with recessive mendelian pigment mutants indicate that isoleucine (and uracil) auxotrophies are recessive traits. A detailed genetic analysis of the ILE401 plants is in

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#### Mouse IgG3 antibodies are highly protective against infection with Streptococcus pneumoniae

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Carbohydrate and protein antigens have been shown to elicit the bulk of their antibodies in mutually exclusive IgG subclasses in humans, mice, rats and horses<sup>1-4</sup>. In the mouse, anti-protein antibodies are primarily of the IgG1 subclass, and anti-carbohydrate antibodies are primarily of the IgG3 subclass<sup>1,2</sup>. We have now demonstrated that mouse IgG3 antibodies to the phosphocholine (PC) determinant of pneumococcal C-carbohydrate<sup>5,6</sup> and to type 3 pneumococcal polysaccharide are highly protective against experimental type 3 pneumococcal infection. This is the first demonstration that antibodies of the IgG3 subclass can protect against bacterial infection.

Previously we have demonstrated that hybridoma IgM anti-PC antibody can protect mice from infection with type 3 Streptococcus pneumoniae<sup>7</sup> strain WU2. Other data (ref. 8 and J. B. Robbins, personal communication) indicate that hybridoma anti-PC antibody can also protect against pneumococcal types 1, 4 and 6A. Much of the anti-PC antibody produced by inbred mice expresses the variable region idiotypic marker, T-15 (ref. 9). Antibodies with this idiotype have virtually identical hypervariable region sequences10, affinity for PC10 and specificity for PC analogues11. We have used protection studies with T-15positive hybridoma antibodies of different isotypes<sup>11</sup> to compare the effects of differences in constant regions without having to consider differences in specificity.

CBA/J mice were infected intravenously (i.v.) with 10° colony-forming units (CFU) of strain WU2 type 3 S. pneumoniae. This dose is 10 times the LD<sub>50</sub> (dose that kills 50% of infected mice). Passively administered IgG3 anti-PC antibody was almost 90-fold more protective against S. pneumoniae on a weight basis than IgM antibody of the same idiotype (Table 1). Similarly, IgG3 anti-type 3 antibody was almost 40 times more protective than IgM anti-type 3 antibody. Protection was not observed for IgA anti-PC antibody, IgM anti-salmonella antibody, or IgG3 anti-levan antibody. Anti-type 3 antibodies were  $\sim$ 6–15-fold more protective by weight than anti-PC antibodies. This was expected as the PC determinants, which form part of the cell wall<sup>6</sup>, should be largely buried beneath the type-specific capsule<sup>12</sup>.

We repeated these studies with  $(CBA/N \times DBA/2)F_1$  mice, which express the X-linked immunodeficiency xid 13,14 extremely low levels of natural anti-PC antibody7,15 and fail to produce anti-type 3 antibody when immunized13. These mice were infected with 150 CFU (~10×LD<sub>50</sub>) of S. pneumoniae strain WU2. IgG3 anti-PC antibodies protected xid mice ~10 times more effectively than IgM anti-PC antibodies, but IgG3 and IgM antibodies to the type 3 capsule showed roughly equal protective ability (Table 2). As with normal animals, anti-type 3 IgM was more effective than anti-PC IgM antibodies; however, IgG3 antibodies of the two specificities provided similar protection. The reasons for the different relative protective abilities of IgG3, IgM, anti-PC and anti-type 3 antibodies in normal and xid mice are unclear, but may be related to differences in immunoresponsive potential and levels of natural serum antibody of the two types of mice, or to the 4-log difference between the infectious doses used in the two studies.

Table 1 Ability of antibodies reactive with pneumococcal cell wall and capsule to protect CBA/J mice against infection with S. pneumoniae strain WU2

Hybridoma or myeloma*	Specificity	Idiotype†	Isotype†	PD <sub>50</sub> (µg per injection)‡
59.6C5	PC	T-15	IgG3	0.6
PC-5-2	PC	T-15	IgM	55
T-15 and	PC	T-15	IgA	>300
H-8				
16.3	SSS-III	?	IgG3	0.1
CA3-1	SSS-III	Unique	IgM	4
CC4-6	SSS-III	Unique	ΙgΑ	>200
J606	Levan	J606	IgG3	>200
ST-1	Salmonella typhimurium	?	IgM	>200

Mice were infected i.v. with 106 CFU of S. pneumoniae7, and protected with intraperitoneal (i.p.) injections of 0.1 ml of the indicated immunoglobulin in doses of 0.02, 0.2, 2.0, 20 or 200 µg (or 100 or 300 µg) 1 h before infection and 1 and 2 days after infection. Injection of diluent alone (0.1% fetal calf serum) was not protective. Antibody and diluent solutions were all filter-sterilized (0.22 µm) before injection. Over 80% of deaths occurred within 4 days of infection. The experiment was discontinued after 10 days.

\* Myeloma antibodies T-15, H-8 and J606 and hybridoma protein 59.6C5 were isolated by affinity chromatography<sup>11,20,25</sup>. Hybridoma proteins 16.3, CA3-1, CC4-6 (ref. 26), ST-1 (ref. 27) and PC-5-2 (a gift from John Kearney) were used as diluted ascites fluids. The amount of antibody in these fluids was quantitated by radioimmunoisotype Comparable dilutions of normal mouse serum were not pro-

† Determination of the idiotypes and isotypes has been described previously<sup>9,11,26,28</sup>; ? indicates unknown idiotype. ‡ PD<sub>50</sub>, dose of passive antibody calculated<sup>29</sup> to protect 50% of the

mice from fatal infection. At least six to eight mice were tested at each

Table 2 Ability of antibodies reactive with pneumococcal cell wall and capsule to protect (CBA/N×DBA/2)F<sub>1</sub> male mice from S. pneumoniae strain WU2

Hybridoma or myeloma	Specificity	Idiotype	Isotype	PD <sub>50</sub> (µg per injection)
59.6C5*	PC	T-15	IgG3	0.6
134.5B11*	PC	T-15	IgG3	0.7
PC-5-2	PC .	T-15	IgM	6
22.1A4°	PC	T-15	IgM	4
140.7C6.2*	PC	T-15	IgM	6
T-15 and	PC	T-15	IgA	>300
H-8			-	
16.3	SSS-III	?	IgG3	0.6
CA3-1	SSS-III	Unique	IgM	0.8
J606	Levan	J606	IgG3	>100
ST-1	Salmonella typhimurium	?	IgM	>200

The mice used here express the X-linked immunodeficiency (xid) defect of CBA/N mice, have no naturally occurring anti-PC anti-body<sup>7,15</sup> and are highly susceptible to pneumococcal infection. Mice were infected i.v. with 100 CFU of S. pneumoniae and protected as described in Table 1 legend.

Anti-PC hybridoma antibodies<sup>11</sup> were produced and isolated as

described in Table 1 legend.

The more efficient protection by IgG3 antibody in normal and xid mice is even more striking considering the fact that divalent IgG3 would be expected to have a much lower avidity than an IgM molecule for identical binding sites. IgM, which is only poorly recognized by Fc receptors 16-18, probably acts by opsonizing S. pneumoniae, using its highly efficient complement fixation<sup>19</sup>. The mechanism of IgG3-mediated killing is not so obvious. Although unaggregated IgG3 does not bind complement Cl (ref. 20), IgG3 anti-streptococcal group A carbohydrate antibody1 can apparently lyse antigen-coated red blood cells in the presence of complement<sup>21</sup>. Thus the C3b receptor of phagocytes may play a part in IgG3-mediated bactericidal activity. Fc receptors for IgG3 (ref. 22) probably contribute to the anti-pneumococcal protection observed here, as they mediate phagocytosis of sheep red blood cells (SRBC) in the presence of IgG3 anti-SRBC antibody<sup>22</sup>.

The possibility that IgG3 may mediate bactericidal activity by an antibody-dependent cellular cytotoxicity is suggested by the fact that human natural killer cells can kill bacteria23, and the recent demonstration that passive IgG3 anti-tumour antibody can protect against tumour growth far better than IgM antibody of the same specificity<sup>24</sup>.

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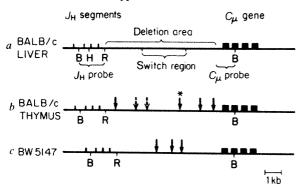
#### The switch region associated with immunoglobulin $C_{\mu}$ genes is DNase I hypersensitive in T lymphocytes

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It is not known whether the antigen-specific receptor of T lymphocytes is encoded by conventional immunoglobulin genes. Several T-cell lines, as well as thymus cells, have been found to contain aberrant IgM mRNAs, but other T-cell lines are apparently negative  $^{1-4}$ . Rearrangement of D and J gene segments which code for portions of immunoglobulin heavy (H) chain variable (V) regions has been observed in some T cells, but no complete V-D-J rearrangements, as required for H gene expression in B cells, have been found<sup>5</sup>. To investigate the chromatin structure around the  $C_{\mu}$  gene in T lymphocytes, we have probed the regions flanking the gene by mild digestion with DNase I. We report here that T-lymphocyte chromatin exhibits sites hypersensitive to DNase I in a region 5' of  $C_{\mu}$ . In B cells, this region has been proposed to be responsible for switching to downstream  $C_{\rm H}$  genes.

It has been observed in other systems that there are sites in chromatin which are hypersensitive to cleavage by DNase I<sup>6-10</sup> The location of such sites can be related to the restriction map of a specific gene. It was postulated that the hypersensitivity occurs at DNA sites which may regulate gene activity in the vicinity 6-10 To determine whether hypersensitive sites are associated with



**Fig. 1** Restriction maps of the region 5' of the  $C_{\mu}$  gene (modified from Liu *et al.* <sup>16</sup>). Deletion area <sup>15–17</sup> and switch region <sup>18–22</sup> were reported for several plasmacytomas to be involved in the deletion of sequences 5' of  $\hat{C}_{\mu}$  and the switch to other  $C_{\rm H}$  genes, respectively. Vertical arrows indicate DNase I-hypersensitive sites (the stippled arrows indicate sites in thymus visible with the  $J_{\rm H}$  probe, but indistinct with the  $C_{\mu}$  probe); \*, site that was only visible with the  $C_{\mu}$  but not with the  $J_{\rm H}$  probe—this site seems also to be hypersensitive in purified DNA (see Fig. 3). The  $J_{\rm H}$  probe is a BamHI-EcoRI fragment cloned by K. Marcu into pBR322 (ref. 15). The  $C_{\mu}$  probe is a BamHI-EcoRI (artificial Eco site) fragment from a germ-line library clone subcloned into pBR322 (R. Near, E. Selsing and U.S., unpublished results).

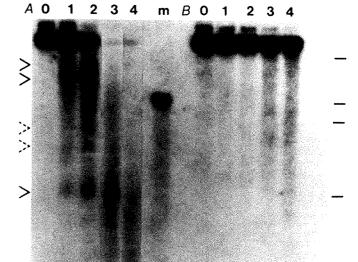


Fig. 2 Southern blots of thymus (A) and liver (B) DNAs from untreated nuclei (track 0) and nuclei treated with DNase I (tracks 1-4) to reduce gradually the size of the DNA to an average of 15-20 kb (track 4)<sup>12</sup>. The DNAs were prepared and digested with BamHI<sup>13</sup>. Southern blots<sup>28</sup> were produced and hybridized with a J<sub>H</sub> probe (see Fig. 1a). Sizing markers are indicated along the right-hand side of the gel-\(\lambda\) phage DNA cut with Aval (8.5, 6.1, 4.8 and 2.2 kb). m, Radiolabelled sizing marker due to the hybridization of the plasmid probe with the 4.6-kb phage  $\lambda$  DNA fragment. Subfragments flanked by a BamHI site and a DNase I-hypersensitive site are indicated by arrowheads. The major hybridization band is the  $J_{\rm H}$ - $C_{\mu}$  gene; the faint fragments above it are due to incomplete digestion by Bam HI.

the  $C_{\mu}$  gene in T lymphocytes, nuclei from thymus cells (>99% T lymphocytes<sup>11</sup>) and liver cells (as a control) were prepared and mildly treated with DNase I12. The range of DNase I concentrations used has been shown to digest active immunoglobulin genes without affecting inactive genes and the bulk of the DNA<sup>12</sup>. DNA was extracted from these nuclei and analysed by restriction enzyme digestion together with Southern filter hybridization to map DNase I-hypersensitive sites8. A restriction map of the  $C_{\mu}$  gene, indicating the enzyme sites used in the analyses, is shown in Fig. 1a.

Liver cells were analysed to provide controls representing a tissue in which immunoglobulin genes are not expressed 12.13. When liver DNA, extracted from untreated nuclei, was digested with BamHI and hybridized with a particular  $J_H$  probe (see Fig. 1a) a single restriction fragment of 9.8 kilobases (kb) was observed (Fig. 2B, track 0). DNAs extracted from liver nuclei treated with increasing amounts of DNase I showed the same Bam HI restriction fragment (Fig. 2B, tracks 1-4). No additional bands were observed, indicating that there are no DNase Ihypersensitive sites in this region of the liver genome.

Thymus DNA, extracted from untreated nuclei, and hybridized with the  $J_{\rm H}$  probe also showed the 9.8-kb Bam HI restriction fragment (Fig. 2A, track 0) seen with liver DNA. This supports the conclusion that in most thymus cells no DNA rearrangement has occurred in the region between  $J_{\rm H}$  and the  $C_{\mu}$ gene. In contrast to the results obtained with liver cells, however, DNase I treatment of thymus nuclei resulted in the appearance of new fragments (indicated by arrows in Fig. 2A). These subfragments must be flanked by a Bam HI site at one end and a DNase I-hypersensitive site at the other. As the  $J_{\rm H}$  probe represents the 5' end of the 9.8-kb  $J_{\rm H}$ - $C_{\mu}$  Bam HI fragment (Fig. 1a), the length of the subfragments produced by DNase I digestion indicates the distance between the BamHI site within the  $J_{\rm H}$  cluster and the DNase I-hypersensitive sites (Fig. 1b) thus allowing localization of the hypersensitive sites in the  $J_{\rm H}$ - $C_{\mu}$ map.

We have confirmed the location of DNase I-hypersensitive sites in thymus chromatin by analysis with a  $C_{\mu}$  probe (see Fig. 3 DNase I-hypersensitive sites in pure DNA. Pure thymus DNA was digested with  $16 \text{ ng ml}^{-1}$  (A) and  $32 \text{ ng ml}^{-1}$  (B) DNase I for 1 min at  $22 \,^{\circ}\text{C}$ , the reaction was stopped with EDTA, the DNA treated with proteinase K and phenol, and then precipitated with ethanol. The size of the DNAs was determined by electrophoresis in agarose gels and found to be slightly degraded to the same size as DNAs 1 and 2 (Fig. 2A) from DNase I-treated thymus nuclei. The DNAs were treated with BamHI, Southern blots 28 prepared and hybridized with the  $C_{\mu}$  probe. The arrowhead indicates a subfragment.

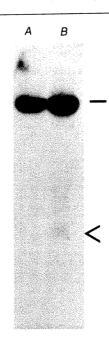


Fig. 1a). This probe represents the 3' end of the 9.8-kb  $J_{\rm H}$ - $C_{\mu}$  1a). This probe represents the 3' end of the 9.8-kb  $J_{\rm H}$ - $C_{\mu}$  BamHI fragment (see Fig. 1a). As before, liver DNA did not show any hypersensitive sites but subfragments were observed for thymus DNA (not shown). The map positions of the major hypersensitive sites in thymus DNA obtained with the  $C_{\mu}$  probe coincided with those obtained using the  $J_{\rm H}$  probe. Some subfragments apparent with the  $J_{\rm H}$  probe (stippled arrows in Figs 1b and 2A) were not discrete using the  $C_{\mu}$  probe (possibly due to a high background in the  $C_{\mu}$  blot).

The hypersensitivity of most of the sites of this genomic area is probably due to the chromatin conformation within thymocyte nuclei. One of the sites, however, may correspond to a nucleotide sequence that is preferentially digested by DNase I, because it is also observed when purified DNA is treated with DNase I (Figs 1 and 3B). It is intriguing that this site is masked in liver chromatin (not shown). The DNA sequence in this region which promotes increased DNase I susceptibility in pure DNA remains to be determined.

The large number of hypersensitive sites observed in thymus chromatin could result from the mixed population of T lymphocytes in the thymus. We have also tested a monoclonal T-cell line, BW5147 (ref. 14), for the presence of DNase I-hypersensitive sites. Mapping of hypersensitive sites in BW5147 is slightly complicated by the fact that in the AKR mouse strain from which this cell line is derived, there is a 0.8-kb deletion between the Bam site within  $C_{\mu}$  and the EcoRI site 5' of the gene (data not shown; see also ref. 15). The deletion is located somewhere within the 'deletion area' marked in Fig. 1a, where deletions in several mouse strains and cell lines have been reported<sup>15-17</sup>. There is apparently no rearrangement between the BamHI sites within  $J_H$  and  $C_\mu$  in BW5147, as the BamHI fragments are of the same size in BW5147 and AKR kidney DNA (data not shown). Using the same strategy as described above, three subfragments were observed in DNase I-digested BW5147 DNA for either the  $C_{\mu}$  (not shown) or the  $J_{\rm H}$  probe (Fig. 4, tracks 1-4). As with thymus DNA, the hypersensitive sites in BW5147 DNA map 5' of  $C_{\mu}$  (Fig. 1c). Obviously there are fewer hypersensitive sites in the DNA of the monoclonal BW5147 cells than in thymus, which may indicate that the complexity of the thymus pattern results from multiple cell populations. We are now exploring this hypothesis further by thymus cell fractionation.

The subfragments produced by DNase I digestion of thymus or BW5147 nuclei are less sharp than restriction fragments; therefore the hypersensitivity is not restricted to a single nucleotide but may span more than 100 base pairs (bp). The same observation has been made for DNase I-hypersensitive

sites associated with immunoglobulin genes in B cells (U.S. and B.A., unpublished data), and with heat-shock genes in  $Droso-phila^{10}$ . The exact location of the  $C_{\mu}$ -associated sites with respect to DNA sequence remains to be determined.

We have not found any hypersensitive sites within 12 kb 3' of the  $C_{\mu}$  gene in thymus or liver cells (data not shown). Together with the fact that liver DNA has no hypersensitive sites 5' of the  $C_{\mu}$  gene, the defined hypersensitive sites 5' of  $C_{\mu}$  in T cells suggest that this area of the genome has been preferentially activated in these cells. This behaviour is analogous to the induced DNase I-hypersensitive sites associated with active globin genes in haematopoietic cells<sup>9</sup>. 'Constitutive' DNase I hypersensitivity of sites in the vicinity of heat-shock genes of Drosophila<sup>8,10</sup> can probably be explained by the fact that these genes have some, albeit low, transcriptional activity at normal temperature<sup>18</sup>.

In B cells the central portion of the intson between  $J_{\rm H}$  and  $C_{\mu}$  has been postulated to be involved in the switching from expression of  $C_{\mu}$  to other  $C_{\rm H}$  genes, because in plasmacytomas which express  $C_{\rm H}$  genes located 3' of  $C_{\mu}$  and which have deleted  $C_{\mu}$ , a 5' portion of the  $J_{\rm H}-C_{\mu}$  intron was retained  $^{19-23}$ . In different cells different switch sites were used. Further analyses of other B cells may show that switch sites are located throughout the region between  $J_{\rm H}$  and  $C_{\mu}$ , as this entire area seems to be prone to deletions  $^{15-17}$ . We have found DNase I-hypersensitive sites in the part of the genome in B lymphocytes that expresses the  $C_{\mu}$  gene (U.S. et al., unpublished data). This may reflect the potential of these cells to switch to another  $C_{\rm H}$  gene.

It is intriguing to speculate that the hypersensitive sites in T cells may be involved in the potential of T cells to 'switch' to the expression of a downstream gene—either one of the  $C_{\rm H}$  genes active in B lymphocytes or a T-cell-specific gene. Other explanations for the hypersensitive sites in T cells must also be considered. Normal T cells and some T-cell lines produce  $C_{\mu}$ -containing RNAs<sup>1.2.4</sup>, which probably do not include correctly joined VDJ complexes (F. Alt, personal communication). Most of these  $\mu$  RNAs have their promoter 5' of  $J_{\rm H}$ , but it cannot be ruled out that some are initiated in the switch region (F. Alt, personal communication). Thus, it is unknown whether any or all of the  $C_{\mu}$ -associated DNase I-hypersensitive sites of T cells mark transcriptional promoters.

Finally, it is possible that the hypersensitive sites in T cells are not directly correlated with any function. First, they may simply

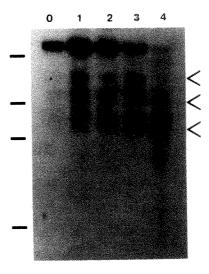


Fig. 4 Southern blot of BW5147 DNA from untreated nuclei (0) and nuclei treated with DNase I (tracks 1-4) to reduce gradually the size of the DNA to an average of 20 kb (ref. 12). BamHI digestion was followed by Southern blotting 13 and hybridization with J<sub>H</sub> probe. Sizing markers indicated along the left-hand margin are λ phage DNA cut with AvaI (see Fig. 2 legend). Subfragments flanked by a BamHI site and a DNase I-hypersensitive site are indicated by arrowheads.

reflect the origin of T cells from a lymphoid precursor common to B and T cells. However, in this context it is surprising that B cells show only one hypersensitive site in this region (U.S. and B.A., unpublished results) compared with at least three in T cells. Second, these sites may have become activated in the course of the activation of some other T-cell-specific gene in the vicinity. This would be analogous to the finding of aberrant rearrangements of immunoglobulin genes in B lymphocytes

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which express some other functionally rearranged immunoglobulin gene<sup>24-27</sup>. The part which B-cell immunoglobulin genes play in T-cell function remains to be elucidated.

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#### Introduction of a rabbit $\beta$ -globin gene into the mouse germ line

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The introduction of cloned foreign genes into cultured mammalian cells<sup>1-7</sup> has been used to identify DNA sequences required for correct transcription in vivo<sup>8-10</sup>. It is not clear, however, to what extent these systems will be useful for an analysis of the sequences necessary for tissue-specific gene expression. A more appropriate approach for such an analysis might be the production of mice that contain a cloned foreign gene in all their cells, throughout development. This could be accomplished by the transfer of a cloned gene into germ-line cells, and the subsequent transmission of that gene to offspring. Previously, SV40 DNA sequences<sup>11</sup> and a cloned HSV-1 thymidine kinase gene<sup>12</sup> have been introduced into somatic tissues of mice by microinjection of the DNAs into blastocysts11 or eggs<sup>12</sup>, but germ-line transmission of these sequences has not been demonstrated. The only foreign DNA sequences which have been transferred into and transmitted by the mouse germline have been exogenous Moloney leukaemia virus genomes introduced by viral infection of early embryos<sup>13</sup>. We now report the introduction of a cloned rabbit DNA fragment containing the adult  $\beta$ -globin gene into the germ-line of mice. We have analysed 24 mice derived from eggs microinjected with this DNA. Nine mice contain the rabbit  $\beta$ -globin gene in liver DNA. and at least four males from this group transmit the gene to a fraction of their progeny.

Fertilized eggs were recovered 3-7 h before first cleavage from (C57BL)6×CBA/H)F1 female mice that had been mated to  $(C57BL/6 \times CBA/H)F_1$  males, and one pronucleus was injected with DNA, using a glass micropipette<sup>12</sup>. The DNA was prepared from a recombinant bacteriophage, λCH4A.RβG2  $(\lambda R\beta G2)$ , which contains the rabbit adult  $\beta$ -globin gene,  $\beta 1$ , and a  $\beta$ -like pseudogene,  $\Psi\beta2$ , in a 19-kilobase pair (kbp) chromosomal DNA fragment<sup>14-16</sup> (see Fig. 1). Most of the  $\lambda R\beta G2$  DNA was in the form of linear monomers but a small fraction (10-20%) consisted of multimers, noncovalently joined by the cohesive ends of  $\lambda$  DNA. Approximately 1 pl of DNA solution, at concentrations ranging from 5 to 50 µg ml<sup>-1</sup>, corresponding to 100-1,000 ARBG2 molecules, was injected into a

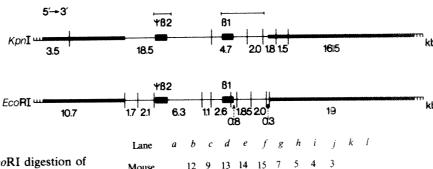
pronucleus. Eggs that survived injection (50%) were transferred to the oviducts of pseudo-pregnant (C57BL/6×CBA/H)F, females<sup>17</sup>, and 15% of them developed to term, yielding a total of 51 live-born mice. Partial hepatectomies were performed on 24 of the mice at 6-8 weeks of age. Total liver DNA was extracted, digested with the restriction endonuclease KpnI and screened for rabbit  $\beta$ -globin sequences by Southern blot hybridization<sup>18</sup>

Figure 2 shows the results for the first 18 mice. Eight of these mice contain sequences in their liver DNA which hybridize to probes for the rabbit  $\beta$ -globin genes,  $\beta 1$  and  $\Psi \beta 2$ . The most intensely hybridizing bands correspond in size to three  $\lambda R\beta G2$ KpnI fragments homologous to the probes (18.5, 4.7 and 2.0 kbp; see Fig. 1). Of six more mice screened (data not shown), one also contains the same  $\lambda R\beta G2$  KpnI fragments. The average number of copies of the rabbit  $\beta$ -globin gene per liver cell in each of the positive mice may be estimated from the intensities of hybridization relative to known amounts of Kpn Idigested  $\lambda R\beta G2$  DNA in parallel lanes. Mice 40 (not shown) and 7 contain 1 or 2 copies, mice 3, 4, 13, 37 and 38, 5-10 copies, and mice 9 and 23, more than 20 copies. Although the sample size is small, there is no obvious correlation between the concentration of the injected DNA and the presence or copy number of  $\lambda R\beta G2$  sequences in these mice.

To determine whether the  $\lambda R\beta G2$  sequences are transmitted through the male germ line, we mated four male mice containing the rabbit sequences with normal CBA/H females. Total DNA was prepared from several whole newborn progeny, restricted with EcoRI and screened for  $\lambda R\beta G2$  sequences using  $^{32}$ P-labelled  $\lambda$ R $\beta$ G2 DNA as a probe. As shown in Fig. 3, all four mice transmitted  $\lambda R\beta G2$  sequences to a fraction of their progeny. These fractions are: one out of four progeny for mice 4 and 23, two out of four for mouse 7, and two out of six for mouse 13. Progeny from the other five positive mice have not yet been analysed. For at least two of the mice transmitting rabbit  $\beta$ globin sequences (mice 4 and 7), the intensity of hybridization to  $\lambda R\beta G2$  is significantly greater for the progeny DNAs than for the parent liver DNAs. One likely explanation is that these mice are chimaeric, and only a fraction of their liver cells contain the  $\lambda R\beta G2$  sequences, whereas all the cells of their progeny contain them. If this is so, the apparent number of copies in liver DNA may underestimate the true copy number in those cells that do contain  $\lambda R\beta G2$  sequences. A further analysis of the tissue distributions and transmission patterns of the rabbit DNA sequences should distinguish between this and alternative explanations.

The results shown in Fig. 3 also provide information about the structure of the  $\lambda R\beta G2$  sequences present in the cells of the

Fig. 1 Restriction maps of  $\lambda R\beta G2$  DNA.  $\lambda R\beta G2$ consists of a 19-kbp rabbit chromosomal DNA fragment (thin line) cloned in a bacteriophage  $\lambda$ -vector (thick lines)<sup>14,24</sup>.  $\beta$ 1 and  $\Psi\beta$ 2 (solid boxes) are, respectively, the adult  $\beta$ -globin gene and a  $\beta$ -like pseudogene<sup>15,16</sup>. The symbols  $\sqcup \sqcup$  and  $\top \sqcap$  represent the single-stranded cohesive ends of the & vector DNA. DNA was isolated from purified phage particles<sup>24</sup> and dissolved in 10 mM Tris pH 7.4, 0.1 mM EDTA for injection into mouse eggs. The bars drawn above the KpnI restriction map depict the regions of ARBG2 that are contained in the probes used in Fig. 2.



Mouse

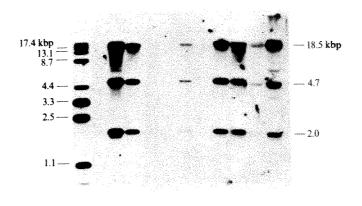
Lane

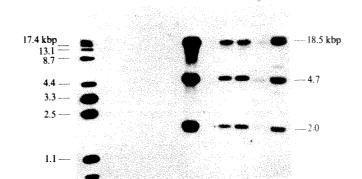
Mouse

positive mice and their progeny. Whereas EcoRI digestion of linear  $\lambda R\beta G2$  DNA generates fragments of 19 and 10.7 kbp, corresponding to the two arms of the  $\lambda CH4A$  vector (see Fig. 1), the mouse DNAs do not contain the 19- and 10.7-kbp fragments; but in every case, a 30-kb band equal in length to the sum of the two & vector arms is seen. In addition, all the bands expected from EcoRI digestion of the rabbit DNA insert in ARBG2 are detected in the DNAs of the positive mice. Together with the results of Fig. 2, these data suggest that the parent liver and progeny DNAs contain intact copies of  $\lambda R\beta G2$ . The 30-kbp EcoRI band in the mouse DNAs is not sensitive to denaturation in conditions that melt the cohesive ends of λ DNA<sup>19</sup> (65 °C, 0.01 M Tris, pH 8), which indicates that the ARBG2 copies exist as either circular or multimeric molecules whose cohesive ends have been covalently joined in the mouse cells.

One possible interpretation of these data is that the  $\lambda R\beta G2$ sequences are integrated into mouse chromosomes in tandem arrays. In this case, the 30-kbp EcoRI fragment in the mouse DNAs would be derived from the joining of the left and right ends of adjacent  $\lambda R\beta G2$  molecules. Linear DNA molecules are rapidly ligated into high molecular weight concatemers following injection into fertilized frog eggs<sup>20</sup>, and DNA-mediated transformation of mammalian cells by the calcium phosphate precipitation method is believed to involve the formation of large DNA consequences before chromosome integration 21,22 large DNA concatemers before chromosome integration Possibly, microinjected  $\lambda R\beta G2$  DNA integrates by a similar mechanism in mouse eggs or early embryos. This interpretation predicts that new EcoRI fragments which hybridize to \(\lambda \text{R} \beta \text{G2}\) should be generated at the ends of each integrated array of ARBG2 molecules. Most of the positive mouse DNAs do, in fact, show one or more new, fainter EcoRI bands in addition to the strong bands that are due to multiple copies of  $\lambda R\beta G2$ . These are visible in several of the lanes of Fig. 3. Mouse 13, for example, shows a new EcoRI fragment about 12 kbp in length (panel e, lane B) which is inherited by both of its positive progeny (panel d, lanes B and F). Mice 7 and 23 (panels  $\hat{b}$  and c) also show new EcoRI fragments which are transmitted to their progeny. The observation that these new bands are inherited is consistent with the prediction that the bands represent junction fragments generated at sites of chromosomal integration. As no more than two or three new bands are seen in either KpnI or EcoRI digests of the positive DNAs, it is unlikely that the mice could contain many individually integrated copies of  $\lambda R\beta G2$ . Although our data support a model of chromosomal integration, they do not formally eliminate other interpretations, such as the autonomous replication and germ-line transmission of free  $\lambda R\beta G2$  circles. We are now attempting to detect integration of the rabbit DNA sequences by in situ hybridization to metaphase chromosomes<sup>22</sup>

In conclusion, we have demonstrated that a large eukaryotic DNA fragment can be introduced at a high frequency into mouse tissues, in the absence of selection, and transmitted through the germ line. Therefore, many strains of mice can now be produced that carry the adult rabbit  $\beta$ -globin gene and its flanking sequences in all their cells. With such strains of mice it will be possible to examine the expression of the rabbit  $\beta$ -globin gene and to investigate whether expression is restricted to specific tissues and/or developmental stages. If the rabbit sequences are integrated into mouse chromosomes, presumably different strains will contain the rabbit genes at different





39

17 18 19 22 23

Fig. 2 Detection of λRβG2 sequences in mouse liver DNAs. Liver tissue samples (0.2-0.4 g) were surgically removed from 6-8-week-old mice, frozen in liquid nitrogen and stored at  $-80\,^{\circ}\text{C}$ . High molecular weight DNA was extracted by the method of Blin and Stafford25 with the following modifications. Powdered frozen tissue was suspended by pipetting in 0.1 M EDTA, 0.05 M Tris pH 8 and subsequently brought to 0.5 M NaCl, 0.5% (v/v) Sarkosyl and 250  $\mu$ g ml<sup>-1</sup> proteinase K. Before the final dialysis step, the DNA was precipitated with ethanol. Yieldsranged from 200 to 800  $\mu$ g of DNA from each liver sample. 10 µg of each DNA was digested with KpnI (New England Biolabs), fractionated by electrophoresis on 0.75% agarose gels and transferred to nitrocellulose filters 18 following cleavage by partial depurination26. The hybridization probe was a mixture of two plasmids, one containing the  $\beta$ 1 gene on a 5.6-kbp Psd fragment and the other containing the  $\Psi\beta2$  gene on a 2.3-kbp EcoRI-BgIII fragment. The regions of  $\lambda R\beta G2$ corresponding to these probes are shown in Fig. 1. Probe DNA was labelled with  $^{32}$ P by nick translation<sup>27</sup> to a specific activity of  $4\times10^8$  d.p.m. per  $\mu$ g and used at a concentration of 7 ng ml $^{-1}$ . Filters were prehybridized for 6 h and hybridized for 18 h essentially as described by Wahl et al. hybridization the filters were washed for 12 h at 66 °C in 2 × SSC, 0.5% SDS. 0.01 M sodium phosphate pH 6.8, 0.05% sodium pyrophosphate and 0.001 M EDTA, and then for 1 h at 55 °C in a 10-fold dilution of the same buffer. In these conditions, no cross-hybridization between the rabbit  $\beta$ globin probe and normal mouse DNA is detected. Lanes a and m, molecular weight markers. Lanes b-j and n-v, Kpnl-digested liver DNA from 18 mice derived from injected eggs. Lanes k and w, 80 pg of Kpnl-digested  $\lambda R\beta G2$ DNA plus 10 µg of salmon sperm DNA. Lanes I and x, 800 pg of Kpnldigested  $\lambda R\beta G2$  DNA plus 10  $\mu g$  of salmon sperm DNA. These amounts correspond to 1 and 10 copies of  $\lambda R\beta G2$  per diploid genome in 10  $\mu g$  of mouse DNA, and the limit of detection on the autoradiographs was 0.1-0.2 copies per diploid genome. The 18.5-, 4.7- and 2.0-kbp fragments of  $\lambda R\beta G2$  hybridize strongly to the probe whereas the 1.8-kbp fragment hybridizes weakly. Other bands visible in the digest of  $\lambda R\beta G2$  (lanes l and x) are partial digestion products

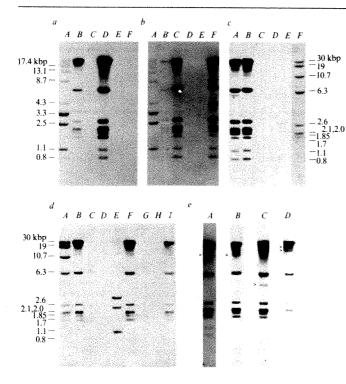


Fig. 3 Transmission of  $\lambda R\beta G2$  sequences through the male germ line. Four male mice containing  $\lambda R\beta G2$  sequences in liver DNA were mated to normal CBA/H females and total DNA was isolated from their whole newborn progeny. Liver DNAs from the four males (10 µg) and total DNAs from broken their progeny (10  $\mu$ g) were digested with EcoRI (Boehringer), fractionated on 0.5% agarose gels and blotted. Filters were hybridized to  $^{32}$ P-labelled  $\lambda R\beta G2$  DNA (2-4×10<sup>8</sup> d.p.m. per  $\mu g$ ; 10 ng ml<sup>-1</sup>). Panel a: lane A, molecular weight markers whose sizes are indicated on the left; lane B, liver DNA from mouse 4; lanes C-F, total DNAs from four progeny of mouse 4. Panel b: lane A, molecular weight markers; lane B, liver DNA from mouse 7; lanes C-F, total DNAs from four progeny of mouse 7. The DNA sample in lane F is incompletely digested by EcoRI. Panel c: lane A, liver DNA from mouse 23; lanes B-E, total DNAs from four progeny of mouse 23; lane F, a mixture of linear monomers and concatemers of ARBG2 digested with EcoRI. The 19- and 10.7 kbp fragments are the λ vector arms of monomeric linear  $\lambda R\beta G2$  (see Fig. 1). The 30-kbp fragment derives from concatemers and consists of the 19- and 10.7-kbp fragments joined by the  $\lambda$  cohesive ends. The smaller fragments are the EcoRI digestion products of the rabbit DNA insert in  $\lambda R\beta G2$ . Panel d: lane A, same as lane F of panel c; lanes B-D and F-H, total DNAs from six progeny of mouse 13; lane E, molecular weight markers; lane I, liver DNA from mouse 13. Panel e: EcoRI-digested liver DNAs from four of the positive mice hybridized to  $^{32}P$ -labelled  $\lambda R\beta G2$ DNA. Each lane is exposed to show one or more new, faint bands not characteristic of  $\lambda R\beta G2$ . Lane A (a longer exposure of lane I, panel d) contains liver DNA from mouse 13 and shows a faint band at which is also visible in two of the progeny of mouse 13 (lanes B and F, panel d). Lane B contains liver DNA from mouse 7 and shows a faint band of  $\sim$ 13 kbp, which is visible in at least one of the progeny of mouse 7 (lane C, panel b). Lane C contains liver DNA from mouse 37 which shows a new band at ~4.5 kbp. Lane D contains liver DNA from mouse 38 which shows two new bands at roughly 15 and 20 kbp. Progeny from mice 37 and 38 have not been analysed.

chromosomal locations. This will allow us to investigate how the host chromosomal environment influences the expression of a foreign gene<sup>2</sup>

Since the submission of our manuscript, chromosomal integration of the  $\lambda R\beta G2$  sequences in mouse 23 has been demonstrated by in situ hybridization to metaphase chromosome spreads prepared from peripheral blood. The probe was 125 I-labelled AR &G2 DNA, and hybridization was performed as described by Robins et al.22. In every metaphase spread examined (19), intense labelling was observed at a site in the middle of one homologue of chromosome 1. This indicates that most if not all of the copies of  $\lambda B\beta G2$  in this mouse are integrated into chromosome 1.

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#### Transcriptional regulation of the prolactin gene by ergocryptine and cyclic AMP

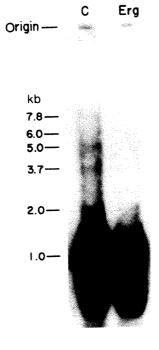
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A large body of evidence suggests that the synthesis<sup>1,2</sup> and secretion3-7 of the pituitary hormone prolactin is inhibited by the hypothalamic hormone dopamine. The finding that dopamine inhibits adenylate cyclase activity of rat pituitary human prolactin-secreting adenoma' suggests that the dopaminergic inhibition of prolactin synthesis may be mediated by decreased levels of cyclic AMP. Recently, the dopaminergic inhibition of prolactin synthesis has been shown to involve decreased concentrations of prolactin mRNA<sup>2,10</sup>. Furthermore, monobutyryl cyclic AMP increases prolactin mRNA levels in pituitary cells treated with the potent dopaminergic agonist ergocryptine11. Such changes in prolactin mRNA levels could involve transcriptional or post-transcriptional events. Here we report that treatment of pituitary cells with ergocryptine leads to rapid inhibition of prolactin gene transcription and that addition of monobutyryl cyclic AMP to ergocryptine-pretreated cells results in a rapid stimulation of prolactin gene transcription.

We first examined the effect of ergocryptine on the levels of nuclear precursors of prolactin mRNA. Previous work has demonstrated the presence of large, potential precursors of prolactin mRNA in pituitary cell nuclei<sup>12</sup>, the largest of these being 7.0 kilobases (kb); the mature prolactin mRNA is 1.0 kb.

Fig. 1 The effect of ergocryptine on possible nuclear precursors of prolactin mRNA. Dispersed pituitary cells were prepared, initially plated in serum-containing medium and then maintained in serum-free Dulbecco's modified Eagle's medium as described previously2, except that the cells were plated in large, 15-cm diameter tissue culture dishes. After 2 days in serumfree medium, half the cultures were treated with 10 nM ergocryptine (Erg) while the controls (C) received only saline. After 24 h, the cells were scraped from the plate in phosphate-buffered saline (PBS) and collected by low-speed centrifugation. The cell pellet was homogenized in 0.25 M sucrose, 20 mM Tris pH 7.9, 5 mM MgCl<sub>2</sub>, 0.1% Triton X-100 using 12 strokes of the tight pestle of a Dounce homogenizer and 2.0 ml of the homogenate were then layered over a sucrose cushion consisting of 1.5 ml of 0.5 M sucrose, 20 mM Tris pH 7.9. 5 mM MgCl<sub>2</sub> and 1.0 ml of 0.3 M sucrose in the same buffer. The sample was then centrifuged at 1,000g for 10 min. The solution above the sucrose cushion was aspirated, the sides of the tube washed with water and the remaining solution decanted.



The nuclear pellet was resuspended in homogenization buffer and centrifuged through sucrose cushions once more. The nuclear pellet was then dissolved in 7 M guanidine HCl, 20 mM Tris pH 7.4, 1 mM EDTA, 1% Sarkosyl using a Dounce homogenizer. The homogenate was layered over 1.5 ml of 5.7 M CsCl, 0.1 M EDTA and centrifuged at 33,000 r.p.m. at 20 °C for 16 h in the SW50.1 rotor. The RNA which pelleted through the CsCl cushion was dissolved in sterile water and precipitated by addition of 0.1 volume 3 M sodium acetate pH 7 and 2.5 volumes of ethanol. Nuclear RNA (8.8  $\mu$ g) from control and ergocryptine-treated cells was denatured by treatment with glyoxal and electrophoresed on a 1.25% agarose gel. The RNA was then transferred to nitrocellulose by blotting as described elsewhere<sup>27</sup>. Prehybridization and hybridization conditions were as described elsewhere<sup>27</sup>. The hybridization probe was a recombinant DNA plasmid, pPRL-2, labelled with <sup>32</sup>P by nick translation <sup>15</sup> to a specific activity of  $2 \times 10^8$  c.p.m. per  $\mu$ g. After hybridization the filter was washed as described elsewhere<sup>27</sup> then exposed to X-ray film in the presence of an intensifying screen. Numbers indicate the sizes of the RNA species in kb.

Hoffman et al.13 have recently detected even larger possible precursors of prolactin mRNA. If the dopaminergic inhibition of prolactin mRNA levels involves effects on the transcription of the prolactin gene, then the concentration of the prolactin mRNA nuclear precursors should decrease. Thus we determined the effect of ergocryptine on prolactin mRNA precursors using techniques similar to those used previously12. Nuclear RNA isolated from ergocryptine-treated and control pituitary cells was denatured with glyoxal, electrophoresed on an agarose gel, then transferred to nitrocellulose. To identify prolactin mRNA species, the filter-immobilized RNA was hybridized to a radiolabelled recombinant DNA plasmid containing the complete prolactin coding sequence<sup>14</sup>. This plasmid, pPRL-2, was labelled *in vitro* with <sup>32</sup>P by nick translation<sup>15</sup>. Using this procedure, multiple, large RNA species containing prolactin sequences were detected in nuclear RNA from control cells (Fig. 1). In ergocryptine-treated cells, there was a considerable decrease in the hybridization of labelled pPRL-2 to all the high-molecular weight species of prolactin mRNA, which suggests that ergocryptine greatly reduced the concentration of prolactin mRNA precursors in these cells. These results suggest that ergocryptine may inhibit transcription of the prolactin gene. However, the RNA transfer technique has at least two limitations for analysis of gene regulation: (1) it is difficult to obtain quantitative information from the procedure-partly due to the fact that different transfers may have different efficiencies and the RNA bands may be better resolved in some transfers than others; (2) the technique only provides information on the concentration of various prolactin mRNA species; it does not rigorously demonstrate changes in synthesis. Therefore, further experiments were performed in which the de novo synthesis of prolactin mRNA sequences was quantitated.

Isolated nuclei provide a useful system for the analysis of transcriptional regulation. In most nuclei transcription systems, RNA synthesis is due primarily to elongation of RNA chains with very little initiation of new RNA chain synthesis 16.17. Therefore, when nuclei are allowed to continue RNA synthesis in vitro, the RNA produced should accurately reflect RNA

		Input	Prolactin mRNA added — (µg)	c.p.m. RNA hybridized		Prolactin cRNA hvbridized	Prolactin mRNA synthesis
Nuclei	α-amanitin	RNA (c.p.m. $\times 10^{-6}$ )		pRL-1	pBR322	nyeraized	(%)
Expt 1							0.055
Pituitary		1.45	0	444	58	48	0.055
Pituitary	+	0.91	0	65	35	47	0.007
HTC cells	-	3.30	0	60	57	40	0.000
Expt 2							
Pituitary		2.10	0	278	69	28	
Pituitary	man.	2.10	0.05	210	65	17	
Pituitary	_	2.10	0.1	137	64	8.8	
Pituitary	www.	2.10	0.5	94	63	4.1	

Table 2 Effects of ergocryptine and monobutyryl cyclic AMP treatment of cultured pituitary cells on prolactin mRNA synthesis by isolated nuclei

	Incorporated UTP	Input	c.p.m. RNA	hybridized	Prolactin cRNA	Prolactin mRNA
Cell treatment	(pmol per μg DNA)	RNA (c.p.m. × 10 <sup>-6</sup> )	pPRL-1	pBR322	hybridized (%)	synthesis (%)
Expt 1						
Controls	0.024	4.83	1,135	106	51	0.042
	0.037	7.04	1,481	148	50	0.038
Ergocryptine	0.032	6.39	246	119	47	0.004
	0.036	6.75	255	92	46	0.005
Expt 2						¥.
Controls	0.030	5.12	795	55	36	0.040
	0.029	4.71	717	77	33	0.041
Ergocryptine	0.033	5.79	278	64	40	0.009
	0.029	5.29	221	50	36	0.009
Ergocryptine + Bt-cAMP	0.045	7.58	783	65	30	0.032
E-goodpane, promin	0.042	7.20	703	81	34	0.025

Monolayer cultures of dispersed pituitary cells were prepared, plated in serum containing medium and then maintained in serum-free Dulbecco's modified Eagle's medium as described in Fig. 1 legend. Cells were treated with 10 nM ergocryptine, 10 nM ergocryptine + 0.5 mM monobutyryl cyclic AMP (Bt-cAMP), or saline (controls) for 24 h then the cells were scraped from the plate in PBS and collected by centrifugation at 500g for 10 min. Isolated nuclei were prepared from the cells as described in Fig. 1 legend, used to synthesize <sup>32</sup>P-RNA, and prolactin mRNA synthesis determined as described in Table 1 legend.

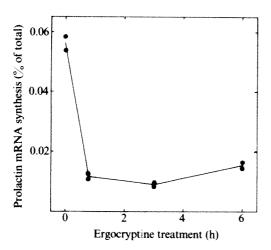


Fig. 2 Time course of ergocryptine effects on prolactin mRNA synthesis. Dispersed pituitary cells from female rats were cultured as described in Fig. 1 legend. After 2 days in serum-free medium, the cells were treated for the time indicated with 10 nM ergocryptine, then scraped from the plates and nuclei prepared. The isolated nuclei were allowed to synthesize RNA and prolactin mRNA synthesis was determined as in Table 1 legend.  $^{32}\text{P-RNA}$  input into each hybridization was  $7{-}10\times10^6$  c.p.m. Points indicate values obtained from duplicate independent assays from the same experiment; the line connects average values.

synthesis which occurred in vivo. The synthesis of a particular RNA can then be determined by hybridization of the RNA to filter-immobilized recombinant DNA containing the sequence of interest. Nonspecific hybridization was determined using filters containing the wild-type plasmid DNA and this value was subtracted from the radioactivity hybridized to filters containing prolactin recombinant DNA. The rate of prolactin mRNA synthesis was calculated by dividing the radioactivity in specific prolactin sequences by the input radioactivity. This procedure was used to examine prolactin mRNA synthesis in pituitary cell and HTC rat hepatoma cell nuclei (Table 1). Prolactin mRNA synthesis was reduced to near background levels by a concentration of  $\alpha$ -amanitin  $(1 \mu g m l^{-1})$  which should inhibit RNA polymerase II (refs 18, 19). Total RNA synthesis was inhibited -40% by this concentration of  $\alpha$ -amanitin. There was no detectable synthesis of prolactin mRNA sequences in nuclei from the HTC cell line. Addition of competitor prolactin mRNA to the hybridization mixture resulted in similar reductions in the hybridization of <sup>32</sup>P-RNA transcripts and prolactin <sup>3</sup>H-cRNA. This demonstrates that the labelled prolactin transcripts result from asymmetric transcription of the sense strand of the prolactin gene. Thus, the synthesis of prolactin mRNA sequences by

isolated nuclei is tissue-specific, asymmetric and mediated by RNA polymerase II.

Treatment of pituitary cells with ergocryptine for 24 h decreased the synthesis of prolactin mRNA sequences by isolated nuclei (Table 2). In one experiment prolactin gene transcription was reduced nearly 10-fold and in a second experiment there was a >4-fold reduction in prolactin mRNA synthesis. Analysis of the time course of the effects of ergocryptine demonstrated that the inhibition of prolactin gene transcription was rapid (Fig. 2). After 45 min of ergocryptine treatment, the earliest time point examined, prolactin mRNA synthesis was reduced almost fivefold and no greater reduction was detected after 3 or 6 h of treatment.

Simultaneous addition of 0.5 mM monobutyryl cyclic AMP partially blocked the ability of ergocryptine to inhibit transcription of the prolactin gene (Table 2). Previous studies have shown that cyclic AMP derivatives have little or no effect by themselves, but that they effectively reverse the dopaminergic inhibition of prolactin synthesis and hence prolactin mRNA levels<sup>11</sup>. Therefore, the effect of monobutyryl cyclic AMP was tested only in cells treated with ergocryptine rather than testing the effect of the cyclic AMP derivative alone. Analysis of the

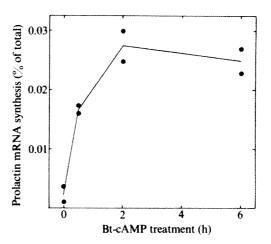


Fig. 3 Time course of monobutyryl cyclic AMP effects on prolactin mRNA synthesis in ergocryptine-pretreated cells. Cultured pituitary cells were prepared as in Fig. 1 legend and after 2 days in serum-free medium they were treated with 10 nM ergocryptine for 24 h. Then 1.0 mM monobutyryl cyclic AMP (Bt-cAMP) was added for the time indicated (the 0 time point received no cyclic AMP) and nuclei prepared from the cells. Transcription and analysis of prolactin mRNA synthesis were as described in Table 1 legend. <sup>32</sup>P-RNA input into each hybridization was 6-8 × 10<sup>6</sup> c.p.m. Points indicate values obtained from duplicate determinations; the line connects average values.

time course of monobutyryl cyclic AMP effects in ergocryptinepretreated cells revealed a rapid stimulation of prolactin gene transcription (Fig. 3). After 30 min, prolactin mRNA synthesis was stimulated about eightfold. Apparently maximal stimulation of >10-fold increases in prolactin gene transcription were detected after 2 and 6 h of cyclic AMP treatment.

The time course and magnitude of ergocryptine effects suggest that the principal mechanism involved in the dopaminergic inhibition of prolactin mRNA accumulation is the inhibition of prolactin gene transcription. Ergocryptine induced a 4- to 10-fold reduction in prolactin gene transcription, which is similar in magnitude to its effects on prolactin synthesis and prolactin mRNA levels2. This inhibition of prolactin transcription is rapid and clearly precedes the changes in prolactin mRNA levels, which decrease much more slowly2.

It seems likely that the dopaminergic regulation of prolactin gene transcription is mediated by cyclic AMP. Previous studies have shown that dopamine agonists inhibit pituitary adenylate cyclase<sup>8.9</sup>, decrease pituitary cyclic AMP levels<sup>11,20,21</sup> and inhibit prolactin synthesis and mRNA accumulation<sup>2,10</sup>. Furthermore, cyclic AMP stimulates prolactin synthesis and prolactin mRNA accumulation in ergocryptine-treated cells11. These studies suggest that in the absence of dopamine, pituitary cells contain high levels of cyclic AMP which stimulate prolactin mRNA accumulation. Thus, dopaminergic treatment decreases cyclic AMP levels, leading to decreases in prolactin mRNA concentrations. The finding that addition of monobutyryl cyclic AMP to ergocryptine-treated cells results in a rapid stimulation of prolactin mRNA synthesis demonstrates that the ability of cyclic AMP to alter prolactin mRNA levels involves effects at the transcriptional level.

Recently, cyclic AMP has been shown to alter the transcription of a gene for a developmentally regulated protein of Dictyostelium discoideum<sup>22</sup>. The finding that cyclic AMP affects transcription of the prolactin gene demonstrates that it can also affect gene transcription in higher eukaryotes. This is particularly interesting in view of the effects of cyclic AMP on prokaryotes in regulating the transcription of specific genes<sup>23</sup>. In Escherichia coli, a cyclic AMP binding protein interacts with specific promoter sites to alter gene transcription. The dopaminergic regulation of prolactin gene transcription may provide a useful model system for further analysis of the role of cyclic AMP in regulating the transcription of eukaryotic genes.

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#### Effects of dexamethasone and cortisone with X-ray irradiation on transformation of C3H 10T<sup>1</sup>/<sub>2</sub> cells

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Hormones can influence carcinogenesis in experimental animals1 and it has been proposed that sex hormones may act like tumour-promoting agents in their ability to enhance the incidence of cancer<sup>2</sup>. On the other hand, it has been observed that the glucocorticoid hormone, dexamethasone, suppresses the enhancement of chemical carcinogenesis in vivo<sup>2</sup> and of transformation in vitro3 brought about by exposure to the tumour-promoting agent, 12-O-tetradecanoyl-phorbol-13acetate (TPA). We report here that the glucocorticoid hormones, cortisone and dexamethasone, do not suppress radiation transformation of C3H  $10T_2^1$  cells in vitro. Indeed, cortisone induces transformation of cells by itself, and increases the yield of radiation-induced transformants in a synergistic fashion.

We used a cell line<sup>4,5</sup> derived from C3H mouse embryos (10T<sub>2</sub>, clone 8) adapted for studies of radiation transformation<sup>6-9</sup>. For the transformation assays, cells were exposed to either 0 or 400 rad of X rays, with or without  $10^{-7}$  M dexamethasone or cortisone (determined in separate experiments to be the highest non-toxic dose). Dexamethasone or cortisone (Sigma) was added to the cultures immediately after irradiation and subsequently added at each medium change for the entire 6-8-week transformation assay.

The assay has been described in detail elsewhere and involves identifying and scoring dense foci of cells in a culture dish. Briefly, type 1 foci represent areas of abnormally dense growth, but with no other deviations from normal cell morphology; cells from these foci are not tumorigenic in mice<sup>6</sup>. Type 2 foci are large and the cells are extensively piled up and moderately stellate in appearance<sup>6</sup>; type 2 cells are tumorigenic in 60-75% of inoculated mice<sup>6</sup>. Type 3 foci are large and contain cells that are very densely piled up and which are highly stellate and show marked criss-crossing and swirling at the border of the focus. Type 3 cells are tumorigenic in 80-100% of inoculated mice<sup>6</sup>. Types 2 and 3 foci were scored as transformants, but tabulated separately.

The results (Table 1) show that cortisone  $(10^{-7} \text{ M})$  by itself is capable of inducing transformation in C3H 10T½ cells, whereas dexamethasone (10<sup>-7</sup> M) is not. When cortisone treatment followed X-ray exposure, the yield of transformants was significantly higher than expected for either agent alone or for the expected additive effect of both agents (using  $\chi^2$  analysis with Yates' continuity correction). Thus, for cells exposed to both cortisone and X rays, there seems to be a synergistic response for the induction of malignant transformation. When radiation exposure was combined with dexamethasone treatment, there was also a higher observed yield of transformants

**Table 1** Effect of cortisone  $(10^{-7} \text{ M})$  and dexamethasone  $(10^{-7} \text{ M})$  on transformation of C3H  $10T_2^1$  cells

		Plating efficiency	Total cells in total no.		otal no. of served foci	which o	f dishes used contained ormants
Group	Treatment	$\tilde{x} + \text{s.e.}$	of dishes	Type 3	Types 2 and 3	Type 3	Types 2 and 3*
а	Controls (no treatment)	$30.9 \pm 6.5$	12,640	0	0	0/40 = 0	0/40 = 0
b	X-ray irradiation (400 rad)	$6.8 \pm 1.9$	29,038	13	35	12/77 = 0.16	26/77 = 0.34
C	Cortisone alone	$31.1 \pm 6.7$	25,270	18	26	11/78 = 0.14	16/78 = 0.21
đ	Dexamethasone alone	$21.8 \pm 5.7$	35,182	1	3	1/78 = 0.01	3/78 = 0.04
$\epsilon$	X-ray irradiation (400 rad) + cortisone	$6.0\pm1.9$	26,070	91	116	41/76 = 0.54	54/76 = 0.71
f	X-ray irradiation (400 rad) + dexamethasone	$6.8 \pm 2.2$	32,449	39	60	23/75 = 0.31	34/75 = 0.45

Details of experimental techniques used in radiation transformation experiments on  $10T_2^1$  cells have been described elsewhere 6-9. Stock cultures were maintained in 60-mm Petri dishes and were passaged by subculturing at a 1:20 dilution every 7 days. The cells used were in passages 9-14. These were grown in a humidified 5% CO<sub>2</sub> atmosphere at 37 °C in Eagle's basal medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Cells were irradiated 24 h after seeding. Plating efficiencies were determined from three plates seeded with a cell density one-fifth that of the plates used for the transformation assay; these cultures were terminated at 10 days. The various toxicities were considered in the design of the experiments. All dishes used for the transformation assay contained ~300 viable cells per dish. Types 2 and 3 foci were scored as transformants, as described by Terzaghi and Little<sup>6</sup>. We have not quantitated our results in terms of 'transformation frequency per surviving cell' because this method should not be used for the malignant transformation of C3H  $10T_2^1$  cells, as described in detail elsewhere<sup>8.9</sup>.

Chi-square analysis (with Yates' continuity correction) of the data for the fraction of dishes used which contained types 2 and 3 foci gave the following results: group a against b, P < 0.001; a against c, P < 0.01; c against e, P < 0.001; b against e, P < 0P < 0.05; b + d against f, P > 0.05. In addition, P > 0.05 was obtained for the data from the three treatment groups when the fraction of dishes containing only type 3 foci was compared. Fisher's exact test (types 2 and 3 foci): a against d, P > 0.05.

than expected for an additive effect of the treatments. However, the yield of transformants after X-ray irradiation and dexamethasone treatment was not significantly greater than the yield of transformants observed for radiation alone or for the expected additive effect of the combined treatments. As similar results were obtained in four separate experiments, these data were pooled (see Table 1).

Mondal and Heidelberger<sup>3</sup> reported that dexamethasone suppressed TPA-enhanced transformation in vitro, and similar results have been reported for TPA-enhanced chemical carcinogenesis in vivo2. However, our results indicate that these glucocorticoid hormones do not suppress in vitro malignant transformation that has been induced by X rays alone. These results suggest that promotion in vivo2 or in vitro9,10 that occurs in the presence of TPA is different from the natural expression of carcinogenesis in vivo or transformation in vitro effected by exposure to a high dose of a carcinogen such as X-ray irradiation. A similar conclusion was reached in other experiments involving the suppressive effects of several protease inhibitors on X-ray transformation with or without enhancement by TPA<sup>11,12</sup>. Scribner and Süss<sup>13</sup> have recently reviewed other evidence for the non-equivalence of complete carcinogenesis, brought about by exposure to a high dose of a carcinogen, and the initiation-promotion carcinogenesis regimen, brought about by a low dose of carcinogen followed by TPA promotion. Cortisone, in that it induced transformation by itself and significantly increased the yield of transformants expected for radiation treatment alone, was similar to  $17\beta$ -oestradiol in its actions on C3H  $10T_2^1$  cells<sup>14</sup>.

Previous quantitative observations on radiation transformation of  $10T_2^1$  cells agree with published data on radiation carcinogenesis in experimental animals, in terms of doseresponse relationships and the effects of factors such as dose rate, linear energy transfer and modifying agents (for review, see ref. 9). These results suggest that the response of  $10T_{\frac{1}{2}}^{\frac{1}{2}}$  cells may be related to the induction of cancer in animals, which is thought to be closely related to carcinogenesis in humans (for review see ref. 15). Thus we believe that our results may have implications for patients treated with a combination of glucocorticoids and cytotoxic agents. For example, patients undergoing total nodal

radiation and combination chemotherapy (which includes glucocorticoid treatment) for Hodgkin's disease have an elevated incidence of secondary leukaemia and lymphoma This has commonly been thought to be caused by exposure to combinations of X rays, alkylating agents and procarbazine; our results also implicate glucocorticoids. In addition, patients who are undergoing chronic immunosuppression by glucocorticoids (organ transplant patients) also have an elevated incidence of Thus, malignancy in transplant patients may be malignancy due not only to immunosuppression itself but also to the agents used to achieve this status. Our results lead us to speculate that when the clinical efficacy of glucocorticoids is similar, dexamethasone may give a decreased risk of tumour formation compared with cortisone.

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Toxicology is the study of the reactions, both adverse and compensatory, of living creatures to noxious materials. The hazards to which man is exposed and the manner in which he reacts to them from the moment of conception and thereafter throughout life is the subject of Human Toxicology. This new journal embraces both animal research, where it has a direct significance for man, and studies on man himself. These studies may be biochemical, physiological, pathological, clinical and epidemiological but will all be designed to increase our understanding of the action of noxious substances in the body and of means to counter their effects. All papers will be subject to peer review.

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Slab gel dryer. New from LKB Instruments is a slab gel dryer which offers accelerated drying times. The LKB 2003 slab gel dryer allows synchronous control of both heat and vacuum sources through a built-in timer and timed outlet to produce fast drying times for all electrophoresis and electrofocusing gels. A 500-W heating element uniformly heats the large  $18 \times 35$  cm drying area to  $80 \pm 2^{\circ}\text{C}$  while a thermal cut-off fuse guards against accidental overheating. The unit is resistant to the acid fumes and organic vapours normally produced during the drying process.

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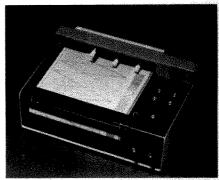
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HPLC guard columns. The new SSI guard column, designed specifically for SSI end fittings, protects the analytical packing from degradation or dissolution by aggressive mobile phases, and from contamination by sample impurities such as particles or precipitated traces of protein. The refillable guard column may be tap-packed with a pellicular packing or slurry-packed with 3, 5 or 10 µm material to match the analytical column. The tip of the guard column is made of material acceptable to HPLC and is designed to seal in the ferrule seat of the end fitting to 5,000 p.s.i. By eliminating any extra tubing or connectors, void volume is kept to an absolute minimum.

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Collection of blood samples. A blood gas Monovette for collection of blood samples for arterial blood gas determinations, is available from Sarstedt, Inc. The unbreakable polypropylene Monovette incorporates a plunger configuration which assures that samples are taken and kept in anaerobic conditions. Preparation with lithium heparin ensures correct anticoagulant concentrations.

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Integrating recorder. The new Linear 200 series integrating recorders are available in single and dual channel models, each with one integrating channel. These recorders incorporate an electro-mechanical integrator. Peak heights are displayed on one channel during integration and recording of the area under that particular curve. Integrator count rates are 3,000, 6,000 and 12,000 counts per min or per h. The chart width is 250 mm; overall limit of error <±1%, chart speeds: 1, 2, 4, 10, 20 and 40 cm min<sup>-1</sup> or cm h<sup>-1</sup>; full-scalesspans of I and 10 mV.

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Ion-exchange TLC. Fixion plates for ion-exchange TLC consist of a strong cation exchange resin, the  $50 \times 8$ , and the  $2 \times 8$ , a strong anion exchange resin. These plates have been used to separate amino acids, nucleic acid components, peptides and pharmaceuticals. A leaflet describing them can be obtained from HPLC Technology. Circle No. 109 on Reader Enquiry Card.

Urine container. Designed for the collection of urine samples over a 24-h period, the Tulip 2500 from L.I.P. has a large built-in funnel and moulded handles that remove discomfort in use and ensure clean collection of the sample on each occasion. The container, when expanded, contains 2.5 litres but is supplied in collapsed form to save storage space. Patient details can be included on the moulded label around the top of the graduated container.

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Electrofocusing. The measurement of pHprofiles in polyacrylamide, the most widely used medium, could previously only be performed by slicing out the appropriate pieces of gel, eluting the ampholyte and measuring the pH of the solution, or by using a soft-surface microelectrode and a pH meter. A new electrophoretic development from BDH Chemicals removes all the difficulties and limitations of these methods: the use of a series of highly purified lyophilized coloured protein standards on electrofocusing media provides a continuous focusing guide, allowing accurate assessment of when the focusing is complete. The gel and standards can be dried to provide a permanent experimental record for comparative purposes. This product is described in a booklet 'Isoelectric Point Markers'

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Variable-wavelength UV monitor. Designed for HPLC applications which require high sensitivity with low noise and drift, the new Bio-Rad 1305 variable wavelength UV monitor provides digital readout of absorbance, transmittance or photodetector output, independent of the recorder. The standard deuterium lamp gives a  $190 - 350 \mu m$  wavelength range, and an optional tungsten lamp extends the range from 350 to 600  $\mu m$ . The monitor features separate electronic and optical modules to conserve bench space, and interchangeable cassette-type flow cells for analytical, preparative and classic opencolumn chromatography.

Circle No. 112 on Reader Enquiry Card.

Bench centrifuge. The new bench centrifuges from Baird and Tatlock feature lid interlock, out-of-balance cut-out and indicator, reinforced chamber, sealed buckets and sealed chamber with outlet vent for ducting. The MkIV auto bench centrifuge has electronically optimized automatic braking and acceleration.

Circle No. 113 on Reader Enquiry Card.

Pocket radiation monitor. Philips have designed a new lightweight pocket radiation monitor for measuring exposure rate and accumulated dose. This compact unit (PW 4514) measures  $\beta$ , X-ray and  $\gamma$ radiation. A four-digit LCD shows both monitoring functions, and a clearly audible alarm sounds if the exposure rate exceeds any of six pre-set levels - 0.5, 2.5, 10, 25, 100 and 250 mR h-1. The alarm continues for a minimum of 5 s so that even momentary excesses are drawn to the user's attention. Maximum exposure rate measurable is 999 mR h-1. The PW 4514 has applications in hospital radiology and isotope laboratories, nuclear power stations and analytical X-ray labs.

Circle No. 114 on Reader Enquiry Card.

Ionization gauge head. Instrument Technology have produced a triode ionization gauge head, suitable for use where pressure readings < 10<sup>-7</sup> torr are not required. In this VIG.71 series, the gauge has an Octal base and can be supplied with push-on connector and safety lead. This gauge head supplements Instrument Technology's VIG.30 series of UHV gauges and, like these, can be connected to a vacuum system, either by direct fusion to glass, or by means of the captured copper gasket and stainless steel flange.

Circle No. 115 on Reader Enquiry Card.

Recording magnetometer. High-sensitivity measurement of the Earth's magnetic field is possible using the G-866 recording magnetometer from EG&G Geometrics. Designed for airborne, marine, land mobile or base-station operations, the microprocessor-controlled G-866 provides resolution to 0.1 gamma and features a built-in digital printer and strip-chart recorder, real-time clock, push-button tuning and sample interval selection ranging from 2 readings per s to 1 reading every 999 s. The chart recorder uses an electrostatic digital plotter mechanism to permanently record data as a dualsensitivity analog chart, annotating the record with exact readings, time, date and scale factors.

Circle No. 116 on Reader Enquiry Card.

Salinometer. A new induction salinometer designed for all marine science applications has been announced by Beckman. The model RS9 is lightweight and provides rapid, accurate measurements of seawater salinity. Salinity determination in the range  $0-51^{\circ}/_{0.0}$  at an accuracy of  $\pm 0.0003^{\circ}/_{0}$ can be made in less than 1 min. The model RS9 can be interfaced with an optional Hewlett Packard model HP-97S calculator, thus eliminating the need to look up salinity in the International Oceanographic Tables. A permanent record of the sample number, salinity, conductivity ratio, temperature and chlorinity is also provided.

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Programmable sample changer. Varian has released a new microcomputer-programmed sample changer, designed primarily for use with atomic absorption spectrophotometers. The PSC-55 may be keyboard programmed to take multiple readings for each sample, rinse at a selected frequency, and perform a re-calibration of slope after a specified number of samples. The sample changer can handle 67 samples, 5 standards, a blank, a re-slope standard and a large reservoir of rinse solution. Samples may be placed in either standard test tubes or disposable microvials. The PSC-55 has a microsampling mode, used when the sample volume is limited or the solution contains large amounts of dissolved solids, by which reliable measurements can be obtained for  $< 100 \,\mu l$ 

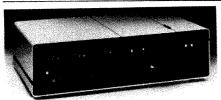
Circle No. 118 on Reader Enquiry Card.

Cryogenic preparation. Hydrated biological specimens for direct scanning electron microscope observation can be prepared using the new SP2000 sputter cryo system from Emscope Laboratories. This system will interface readily to any scanning electron microscope and includes facilities for freezing, fracturing, sputtercoating, sublimation and etching, which allows it to be used in all areas of routine and research biological scanning EM.

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Hot agar dispenser. Using the bubble eliminator from Kraft Apparatus, bubble-free agar can be dispensed accurately. The pumping head features total ball-bearing construction and variable volumes per unit time can be dispensed using the variable speed motor. Delivered volume is adjustable from 2 to 575 ml with 1.5 reproducibility.

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lonized calcium analyser. A computerized system that measures ionized calcium and pH for 125-µl samples of whole blood, plasma or serum has been introduced by Radiometer. In addition to these direct measurements, the ICA1 calculates ionized calcium at pH 7.4. The measurements are based on ion-selective electrodes. A calcium-ion-sensitive electrode a pH electrode and a common reference electrode are all mounted in a measuring chamber, maintained at 37°C. The ICA1 has a built-in monitoring system: all parts and functions are checked by the computer.

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Tracking animal movements. The Videomex system from Columbus Instruments consists of a vidicon TV camera and Apple-II personal computer, and allows automatic tracking of animal movements within the observation field of the camera. The x-y coordinates of the animal are computed and plotted on a computer printout. Programs available allow computation of the distance the animal travels, number of small and large movements, and time spent moving and resting. Animal rotational behaviour is detected automatically - this is an important test of pharmacological compounds with dopaminergic activity. The number of clockwise and counterclockwise turns, as well as incomplete turns, of the animal are counted.

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Ion implantation system. Varian has developed a new multi-purpose ion-implanter system — the DF-3000 — evolved from the 200-DF5, the industry standard for medium-current ion implanters. The new system, with up to 300% increase in scanned beam current, reduces the time required to achieve highly uniform implants. In addition, the DF-3000 reduces problems of wafer contamination due to particulates common in earlier ion-implantation equipment. The system offers scanned beam currents of 1,500 μA for P and 1,250 μA for As.

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#### **NEW LITERATURE**

Test and measuring instruments. A range of oscilloscopes, chart recorders, frequency counters and logic analysers is described in a new catalogue from Enertec Instrumentation Ltd. Circle 150.

**Chromatography.** Additions to the Dionex range of chromatography products are listed in a new catalogue. Circle 149.

Reactors and pressure vessels. The complete Parr line of laboratory reactors and pressure vessels is described in a new catalogue. Information on operating pressures, temperatures, construction materials and accessories is included. Circle148.

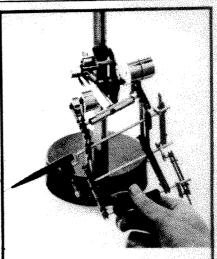
**pH** monitoring. New literature from Beckman Instruments describes their **pH** monitoring system, designed for waste water applications. Circle 147.

**Electrophoresis chamber.** The new 600 electrophoresis chamber is described in a brochure from Shandon. Circle 146.

**Radioactive solutions.** Amersham have produced a standardized radioactive solutions catalogue. Circle 145.

Hybridization technology. Schleicher & Schuell, Inc. have brought out 'Products for Nucleic Acid and Protein Research', which surveys solid-support hybridization techniques. Circle 144.

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BERESTYCKI, H. and BREZIS, H. (eds). Recent Contributions to Nonlinear Partial Differential Equations. Research Notes in Mathematics, 50. Pp.226. Flexi ISBN 0-273-08492-5. (Pitman: 1981.) £9.95.

DUFF, I.S. (ed.) Sparse Matrices and their Uses. Based on the Proceedings of the IMA Numerical Analysis Group Conference, held at the University of Reading, July

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HASSARD, B.D., KAZARINOFF, N.D. and WAN, Y-H. Theory and Applications of Hopf Bifurcation. London Mathematical Society Lecture Note ries, 41. Pbk ISBN 0-521-23158-2. (Cambridge University Press: 1981.) Pbk £15.

KNOPS, R.J. (ed.). Trends in Applications of Pure Mathematics to Mechanics. Vol.III. Papers presented at a Symposium at Heriot-Watt University, September 1979. Pp.234. ISBN 0-273-08487-9. (Pitman: 1981.) £25.

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LIPSON, J.D. Elements of Algebra and Algebraic Computing. Pp.342. ISBN 0-201-04115-4. (Addison-Wesley: 1981.) \$34.50.

RUCKLE, W.H. Sequence Spaces. Research Notes in Mathematics, 49. Pp.197. Flexi ISBN 0-273-08507-7. (Pitman: 1981.) £8.50.

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PASACHOFF, J.M. Contemporary Astronomy. 2nd Edn. Pp.545. ISBN 0-03-057861-2. (Holt, Rinehart & Winston: 1981.) Np.

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BURKE, J.J., MEHRABIAN, R. and WEISS, V. (eds). Advances in Metal Processing. Proceedings of the 25th Sagamore Army Materials Research Conference, held at the Sagamore Hotel at Bolton Landing, Lake George, New York, July 1978. Pp.388. ISBN 0-306-40651-9. (Plenum: 1981.) \$45.

DREW, T.B. et al. (eds). Advances in Chemical Engineering, Vol.11. Pp.452. ISBN 0-12-008511-9. (Academic: 1981.) \$58.

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PRESTAYKO, A.W. et al. (eds). Nitrosoureas: Current Status and New Developments. Pp.416. ISBN 0-12-565060-4. (Academic: 1980.) \$30.

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#### CHRISTIE HOSPITAL AND **HOLT RADIUM INSTITUTE**

PATERSON LABORATORIES

#### **MEDICAL LABORATORY** SCIENTIFIC OFFICER

required for work in a Histopathology Laboratory providing a service to biological research units.

Qualifications: ONC or HNC in Medical Laboratory Sciences (Histopathology) or equivalent experience.

Salary: £2,725 to £4,350 or £4,958 to £6,993 pa according to age, qualifications and experience. Day release available where required.

Applications to Laboratory Administrator, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, quoting reference PL/81/6 and enclosing SAE. Closing date: 16th November 1981. (9843)A

#### POST DOCTORAL RESEARCH ASSOCIATE

Position is available immediately to study the molecular basis of surface glycoprotein gene expression in African trypanosomes and to initiate studies on surface antigen gene expression in leishmania parasites. Only candidates with research experience in biochemistry or preferably molecular biology will be considered. Position for up to two and a half years with a negotiable salary range of \$13,000 to \$15,000.

Please send all inquiries and/or curriculum vitae and names of two references to: Dr Kenneth B Marcu Biochemistry Department, SUNY Stony Brook, Stony Brook, NY 11794. SUNY Stony Brook is an equal opportunity/affirmative action employer. AK 205

(NW043)A

#### INSTITUTE OF CHILD HEALTH University of London

#### PRE OR POST DOCTORAL RESEARCH SCIENTIST

required as soon as possible for three year project to investigate the immunological factors controlling tolerance to food antigens and their disturbance in diseases caused by food allergy. Initial salary within the range £5,285 — £6,475 plus £967 London Weighting per annum (University scales 1A or 1B for Research and Analogous Staff). Opportunity to register for a higher degree, if required.

Further information from Dr R J Levinsky or Dr M W Turner, Immunology Department, Institute of Child Health, 30 Guilford Street, London WC1N 1EH. Tel: 01-242 9789 ext 133 or 130. (9840)A

#### FRIEDRICH-MIESCHER-LABORATORIUM OF THE MAX-PLANCK-GESELLSCHAFT TUEBINGEN, GERMANY

research associate position is available for at least two years in a new independent group working on aspects of the cytoskeleton, the cell surface, and cell secretion (see PNAS 77, 4108 (1979); Cell 22, 555 (1980), TIBS 6, 234 (1981)).

As techniques, monoclonal antibodies and microinjection with glass capillaries are used. The salary will be according to a German BAT Ila/Ib position and be about DM 30,000 to 40,00 per year.

Applications with references (incl. number) should be sent to Dr Walter Birchmeier, Laboratorium für Biochemie, Federal Technical University (ETH), CH-8092 Zürich, Switzerland. (W484)A

# Sr. Research Biochemical Engineers

You've probably heard that Merck Sharp & Dohme Research Laboratories has one the largest R&D budgets in the pharmaceutical industry. For innovative engineers and scientists, that translates into exceptional opportunities to achieve new breakthroughs. Both in pharmaceutical and biological products. And in your career.

We're now seeking two scientists/engineers with PhD degrees and postdoctoral/industrial experience in these areas:

- INDUSTRIAL MICROBIOLOGY
- **FERMENTATION TECHNOLOGY**
- EUKARYOTIC GENETIC ENGINEERING

- IMMUNOCHEMISTRY
- **BIOENGINEERING**

Positions will involve process research, development and scale-up of microbial fermentations, cell culture systems and macromolecular product isolations. Specific requirements include PhD degree (or equivalent) in Chemical Engineering/Biochemistry/Microbiology, with at least one degree in Chemical/Biochemical Engineering. Demonstrated leadership capability is essential. Postdoctoral work preferred; as is strong background in biochemistry, virology, microbiology, separation engineering or recombinant DNA microorganisms.

Growth opportunities are complemented by excellent salaries and outstanding benefits. Send résumé with salary requirements to Ms. Johanna Y. Zeltner, Ref. NA-1.



P.O. Box 2000, Rahway, New Jersey 07065

An equal opportunity employer m/f

(NW035)A

# Radio Astronomy Research & Development Overseas

Goal-orientated R&D work on a 3-year contract or permanent basis with a world leader in South Africa.

The National Institute for Telecommunications Research carries out research into all aspects of radio and its applications. We have been responsible for a number of significant technological breakthroughs including 2 world 'firsts': inventing the Tellurometer and subsequently developing an infra-red distance measuring system. Achievements that we attribute largely to our policy of actively encouraging initiative at all levels.

The Radio Astronomy Observatory is situated in a pleasant, protected valley about 35 miles from Johannesburg. The telescope has a 26 metre diameter and operates under

computer control.

With minimum supervision and maximum observation time, you will be able to study radio emissions from astronomical objects, analyse/interpret results, give occasional lectures and possibly assist with the instrumentation and computer software development. Both team and individual

project work are involved.

Consequently you should possess at least a BSc (Hons) with a physics, astronomy, computing or electronics background. These posts will prove very attractive to those of you currently holding posts with established radio astronomy observatories.



The CSIR was established to develop the Republic's research-oriented manpower, material resources and national/community services through 4,500 personnel employed at 19 research laboratories and institutes nationwide.

**COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH** 

Whether you choose to join us on a 3-year contract (renewable) or permanent basis, salaries are negotiable and these will be augmented by the Republic's high standard of living at relatively low cost (at least 30% below that of the UK) plus the following benefits • free air passages (both ways for contract appointees) • assistance with removal costs

- settling-in allowance generous house purchase scheme, where applicable annual bonus equal to one month's salary medical aid, pension/life assurance schemes
- outstanding schooling (English-medium), sports and recreational facilities, etc.

In addition, researchers' findings may be used as a basis for Master's and Doctoral dissertations.

For an application form, either ring 01-242 1766 during normal office hours or send brief personal and career details

in the UK.

(including phone number) to the Scientific Counsellor, Chichester House, 278 High Holborn, London WC1V 7HE, quoting reference 11/1/686 clearly on your envelope. All interviews will be conducted

(9838)A

#### CAWTHRON INSTITUTE NELSON, NEW ZEALAND

invites applications for the position of

## **Research Microbiologist**

to work on microbial fermentation and the processes involved in transforming biological products and wastes into other compounds.

A qualified research scientist with training and experience in biotechnology and microbial physiology and biochemistry will be given wide scope to investigate innovative ways of converting materials and evolving new processes which might lead to the development of interesting and useful compounds e.g. fuels, pharmaceuticals, enriched feeds, foods, biochemicals etc.

The position requires a good researcher with the drive, initiative, and versatility to develop a programme of investigation.

The appointee will join a small research group of microbiologists engaged in laboratory and field investigations of a number of microbial processes e.g. methanogenesis, nitrification, denitrification, phosphate mobilisation, and fermentation.

The appointment will be for an initial period of three years with the possibility of reappointment.

Salary will depend on age and experience and will be within the range NZ \$15,23,000.

Fares to Nelson and reasonable expenses for transport and personal goods and effects will be paid to appointee and dependent family.

Applications (3 copies) containing full curriculum vitae, a recent photograph and the names of three referees should reach the Director, Cawthron Institute, Box 175, Nelson, New Zealand not later than 20 November 1981. (W483)A

# UNIVERSITY OF NEWCASTLE UPON TYNE THE DENTAL SCHOOL LECTURER IN ORAL PHYSIOLOGY

Applications are invited for the post of LECTURER IN ORAL PHYSIOLOGY

in the new Department of Oral Biology. The post is open to candidates with or without dental qualifications who are interested in physiological or biochemical research, for which there are excellent facilities in the Department. The successful candidate will be required to undertake undergraduate and postgraduate teaching in oral physiology and to participate in research

Salary will be at an appropriate point, according to age, qualifications and experience, on the Lecturers' Pre-Clinical scale £8,105 — £12,860 per annum for dentally qualified candidates or on the Lecturers' scale £6,070 — £12,860 per annum without dental qualifications.

Further particulars may be obtained from the Deputy Registrar (FP), The University, 6 Kensington Terrace, Newcastle upon Tyne NEI 7RU, with whom applications (3 copies), together with the names and addresses of three referees, should be lodged not later than 21st November 1981. Please quote reference N.

(9821)A

INSTITUTE of Cancer Research: A Post-Doctoral Fellow is required for the Division of Cell and Molecular Biology, Chester Beatty Research Institute, Fulham Road, SW3, to work on the isolation and characterisation of eukaryotic chromosomal proteins. Experience in amino acid sequencing would be an advantage but general experience in protein chemistry would be acceptable. The post is for two years in the first instance with a possible extension of one year. Starting salary in range £6,070 — £7,700 pa plus London Allowance of £967 pa. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting Ref. 300/G/12. (9827)A

#### CHURCHILL HOSPITAL Medical Genetics Dept, Old Rd, Headington SENIOR SCIENTIFIC OFFICER

A vacancy occurs in the Department of Medical Genetics from March 1982 for a Senior Scientific Officer. The successful applicant will have an appropriate degree, plus several years experience in human cytogenetics. The salary range is from £8,134 to £10.516.

In the first instance send for an application form to Dr R H Lindenbaum, Department of Medical Genetics, Old Rd, Headington, Oxford OX37LE. (9830)A



# IMMUNOLOGY PHARMACOLOGY MOLECULAR BIOLOGY RESEARCH

SmithKline, a leader in pharmaceutical research, is undertaking a major expansion of its research division. Applications are invited from research scientists (Ph.D./ M.D./ D.V.M./ M.S./ B.S.) to join interdisciplinary research teams to participate in the development of new therapeutic agents in a stimulating environment that offers significant opportunities for innovative research.

Our scientists are encouraged to publish, to hold adjunct academic posts and to interact fully with the national and international scientific community. A new, superbly equipped \$27 million research facility is under construction at our Upper Merion research campus in suburban Philadelphia and will be completed in 1982. This represents the first stage of a \$200 million R&D facility under construction at this site for completion by 1985.

We are seeking outstanding individuals at all seniority levels who have expertise in one or more of the following subjects:

# **IMMUNOREGULATION**

(ref: MB-1)

identification of lymphoid cell subsets / cell sorting /lymphokines/macrophage physiology/auto-immune diseases

# HYBRIDOMA TECHNOLOGY (ref: MB-2)

hybridoma production / characterization of monoclonal antibodies/ cell sorting

# MOLECULAR GENETICS (ref: MB-3)

DNA sequencing/nucleic acid enzymology/gene construction/gene cloning and expression in Bacillus, actinomycetes, yeast and mammalian systems/promotor regulation/gene amplification

# FERMENTATION TECHNOLOGY

(ref: MB-4)

whole cell and enzyme immobilization / fermentation methods/biochemical and fermentation process engineering

# **CELL BIOLOGY**

(ref: MB-5)

large scale mammalian cell culture 7 cell fusion / microinjection/bone marrow and tumor stem cell cloning/platelet physiology/mutagenesis/electronmicroscopy/subcellular fractionation

# ENZYMOLOGY AND PROTEIN CHEMISTRY

(ref: MB-6)

protein sequencing/peptide synthesis/ isolation and purification of lymphokines and cytokines / macromolecular separation / radioimmunoassays/microanalysis/instrumentation development

# RECEPTOR BIOLOGY

(ref: MB-7)

receptor isolation, characterization and reconstitution/pharmokinetics/radicimmune assays/biology of dopaminergic, adrenergic, serotoninergic, histaminergic and peptidergic receptor systems

# MOLECULAR PHARMACOLOGY

(ref: MB-8)

drug-chromatin interactions/nuclec-cytoplasmic interactions/regulation of mac@omolecular synthesis/advanced biophysical instrumentation/cell fractionation

We offer excellent compensation and benefits, an attractive relocation policy and a work environment that offers substantial opportunity for personal and professional advancement. For confidential consideration, send C.V., salary expectations and names of three references, citing the appropriate reference number listed above to: Mr. Walter B. Flagg.

# **SmithKline**

P.O. Box 7929, 1558 Spring Garden Street Philadelphia, PA 19101 We are an equal opportunity employer, M/F/H/V

(NW030)A

# ADMIRALTY UNDERWATER WEAPONS ESTABLISHMENT Portland, Dorset

A vacancy exists for a

# Junior or Senior Research Fellow

to study the application of MACHINE INTELLIGENCE techniques, including EXPERT SYSTEMS and FUZZY REASONING, to naval problems. The successful applicant will work at Portland, but will be expected to co-operate closely with researchers at the University of Bristol and elsewhere. Candidates will be expected to demonstrate an excellent record of research. It might be possible for a suitable applicant without a PhD to use this work as the basis for a submission. The appointment will be for a fixed period of up to three years.

Depending on qualifications and experience, the stipend will be in one of the following ranges:

JUNIOR RESEARCH FELLOW £6,302 — £7,507 (Minimum of two years post-graduate research experience, not necessarily in a directly related subject).

SENIOR RESEARCH FELLOW £8,596 to £9,676 (Minimum of three years postgraduate research experience in a directly related subject).

All candidates must have a first or second class honours degree.

Application forms may be obtained from the Scientific Personnel Officer, Admiralty Underwater Weapons Establishment, Portland, Dorset DT5 2JS. Telephone: Portland (0305) 820381 ext 3433.

Please quote reference: AX787/U/151.708/AXS. Closing date for applications 27th November 1981. (9822)A

# Biological Scientist

# **Fruit Preservation**

Tate & Lyle are developing coatings for fruit as preservation and storage aids. This work has created a need for an honours graduate with 3-5 years postgraduate experience preferably in post harvest physiology which could include Ph.D. and/or industrial work. The successful candidate will be qualified in a biological science and he/she will join a multi-disciplinary team engaged in studying means of controlling the ripening of fruit. The group conducts fruit trials and looks at biochemical and biophysical effects related to the ripening process. Salary and benefits are competitive. Please apply with CV to:

Salary and benefits are apply with CV to: Personnel Manager, Tate & Lyle Group Research & Development, P.O. Box 68, Reading RG6 2BX.

ent, (9848)A

Tate & Lyle

# UNIVERSITY OF GUELPH WILDLIFE BIOLOGIST

The Department of Zoology offers a faculty position at the assistant or associate professor rank for a wildlife biologist. The successful applicant will be expected to teach undergraduate courses including wildlife management and biology.

Candidates must have at least 2 years post-doctoral experience and will be expected to develop a research and graduate program.

Position to be filled by April 1st, 1982 subject to final budgetary approval.

Application, including a curriculum vitae with the names of three referees should be sent to: The Chairman, Department of Zoology, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1.

Only those applicants legally eligible to work in Canada need apply. (NW034)A

# UNIVERSITY OF TORONTO DEPARTMENT OF MEDICAL GENETICS ASSISTANT OR ASSOCIATE

### PROFESSORS POSTDOCTORAL FELLOWS

The University of Toronto, Department of Medical Genetics, invites applications for two positions open at either the Assistant or Associate Professor level. One position requires a background in animal models of metastasis and quantitative assay of metastatic potential. The other position requires a background in the isolation and structural identification of cell surface glycopeptides and oligosaccharides. The appointees will be expected to conduct independent research programmes in their area of expertise, as applied to the investigation of the molecular basis of metastasis. This research will be conducted within a multidisciplinary group funded for five years by a Terry Fox Special Initiatives Grant from the National Cancer Institute of Canada. Start-up funds will be provided for personnel, equipment and supplies. Opportunities for eventual permanent staff positions are excellent. Candidates must have a PhD or equivalent, and appropriate post-doctoral training. Salary and rank will be commensurate qualifications and experience. with

Three positions within the above research group are open for post-doctoral fellows with backgrounds in either of the two previously mentioned areas, or in somatic cell genetics. Salary support for three years is available for each of these positions.

Closing date for all applications is December 31, 1981. Positions will take effect April 1, 1982 or as soon as possible thereafter. Applicants are asked to submit a curriculum vitae with a list of publications, and the names of three referees to either: Dr Harry Schachter, Department of Biochemistry, Research Institute, Hospital for Sick Children, Toronto, Ontario, M5G 1X8; OR Dr Jeremy Carver, Department of Medical Genetics, Medical Sciences Building, University of Toronto, Toronto, Ontario, M5S 1A8. (NW031)A

# RESEARCH TECHNICIAN

Applications are invited from individuals to develop *in vitro* culture techniques for entomophilic nematodes and novel, biochemical methods for nematode identification. Qualifications: experience in the culture and taxonomy of entomophilic nematodes; familiarity with modern taxonomic techniques and electron microscopy; postdoctoral experience. Starting salary \$16,000 per year.

Applicants should send an outline of their research interests, a curriculum vitae and the names and addresses of two referees, as soon as possible to: Dr John M Webster, Department of Biological Sciences, Simon Fraser University, Burnaby/Vancouver, BC Canada V5A 1S6.

(NW021)A

### INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES Nairobi Kenya

Applications are invited from suitably qualified candidates for

### POSTDOCTORAL FELLOW Ref. No. PDF/L3/81/1

A position is available from January 1, 1982 for two years to improve laboratory technology in the study of trypanocidal drugs in vitro. Applicants must have PhD (or equivalent) in Science or Pharmacology, practical experience in cultivating African trypanosomes and various mammalian cells in vitro and solid knowledge of organic chemistry and be capable to apply above background to the project without further training on the cultivation of trypanosomes. The successful candidate will work on the joint programme with the collaboration of Cell Biology Laboratory (ILRAD), Kenya Trypanosomiasis Research Centre and GTZ-supported Chemotherapy of Trypanosomiasis Research Project/Ministry of Agriculture. This is an international post and the salary will be paid in US\$.

Applications identified with Ref: No. including CV, summary of PhD thesis, and three references should be sent by 19 November 1981 to:

CHIEF PERSONNEL OFFICER,

I.L.R.A.D., P.O. Box 30709, NAIROBI, Kenya.

(W465)A

# AGRICULTURAL RESEARCH COUNCIL VETERINARIAN

Required to join a group studying possible hazards associated with new developments of husbandry procedures. The post will provide veterinary expertise to existing research projects which include the study of the use of anabolic agents in farm animals. In particular, the applicant will investigate the endocrinological mechanisms which may control mammary development in the dairy heifer, and the relationship between fatty liver and infertility in the cow.

Salary: Veterinary Research Officer scale £10,398 × 5 to \$13,448 p.a. (depending on age and experience). Non-contributory pension

Qualifications: Applicants must be members of the Royal College of Veterinary Surgeons, with at least two years post-graduate experience, preferably in endocrinology.

The Institute is located in pleasant rural surroundings in an estate of 2,000 acres and has a staff of 295.

Application forms from: The Secretary, Institute for Research on Animal Diseases, Compton, Nr Newbury, Berks RG16 0NN, quoting reference 470. Closing date: 21/11/81.

# THE ST GEORGE HOSPITAL DIRECTOR OF PATHOLOGY

Senior Staff Specialist in Immunopathology with conjoint appointment as

# CLINICAL PROFESSOR OR CLINICAL ASSOCIATE **PROFESSOR**

School of Pathology University of NSW

Applications are invited from medical graduates with senior qualifications in pathology for the above position. The successful applicant will be responsible for participating in and supervising immunopathological services as well as directing the overall diagnostic laboratory procedures for a 600 bed Teaching Hospital of the University of NSW. The Department of Pathology is currently staffed by six full-time Specialists. It is intended that the appointee would be offered the title of Clinical Professor (Conjoint) or Clinical Associate Professor (Conjoint) and as such would be responsible to the University of New South Wales for teaching in the School of Pathology, with access to the facilities of that School.

Salary is at Senior Staff Specialist rate, \$45,695 per annum and an appropriate administrative allowance is payable. Conditions in accordance with the Medical Officers' Hospital Specialist (State) Award, include three months paid study leave each five years of service. Limited right of private practice in accordance with procedures laid down by the Health Commission is also available.

Written applications, including the names and addresses of three referees, close with the Chief Executive Officer The St George Hospital, Kogarah, NSW 2217, on 31st December, 1981. Enquiries concerning hospital duties should be directed to the Director of Medical Services phone (02) 588 1111, and on University matters to Professor A Lykke (02) 662 2771. (W477)A

### THE CASWELL SILVER DISTINGUISHED PROFESSORSHIP IN GEOLOGY THE UNIVERSITY OF **NEW MEXICO**

The Department of Geology of the University of New Mexico is pleased to invite nominations or applications for the Caswell Silver Distinguished Professorship in Geology. This endowed professorship shall be awarded for periods of up to two years to earth scientists of distinguised accomplishment and international reputation. The international reputation. The professorship may be held by scientists of all specialties of the earth sciences in the broadest sense, and the major criterion for selection is that the individual be an active, productive leader in his or her field of research. The recipient must carry out a vigorous reserch program while in residence at UNM. The recipient is expected to interact with the faculty and students of the Department and to provide one or more seminars, in an advanced topic of his/her choice, during each academic year. The Foundation will provide unusually advantageous remuneration commensurate with the distinguished nature of the appointment. In addition, a generous allocation for travel and operating expenses (to include secretarial support, analytical services in department laboratories, use of field vehicles, and preparation of manuscripts) will be provided.

Applications or nominations should includes a detailed résumé and brief statement of major research accomplishments. Applications or nominations should be forwarded to: Rodney C. Ewing,

Chairman, Department of Geology, University of New Mexico, Albuquerque,

New Mexico 87131. The deadline for applications is January 1, 1982. The Caswell Silver Foundation is an equal opportunity employer. (NW032)A employer.

# Synchrotron Radiation Research SURFACE SCIENTIST The Synchrotron Radiation Source at the Daresbury

Laboratory is the world's first high energy (2 GeV) storage ring specifically designed to provide a large number of synchrotron radiation beams. A Surface Scientist is required to assist Laboratory staff

and University users in designing, setting up and maintaining beam lines and experimental apparatus and running experiments in the general area of surface science. The apparatus involved will include two electron spectrometers for angularly-resolved photo-emission and one for x-ray absorption studies on surfaces, together with their associated optical monochromators and minicomputerbased data acquisition systems. There will be opportunities to collaborate in research projects with Laboratory and University staff.

Candidates should have a good honours degree in an appropriate discipline, post-graduate experience in surface science and specific practical expertise in some or all of the fields of ultra-high-vacuum, electronics, vacuum ultraviolet/ x-ray optics and microcomputer programming.

An appointment will be made at Scientific Officer/ Higher Scientific Officer level on the salary scale £5,176 to £6,964/£6,530 to £8,589 per annum, subject to qualifications and experience. The appointment will be superannuable. There is a generous leave allowance and a flexible

working hours scheme. CLOSING DATE: 20th November, 1981. For further information please write or telephone Warrington (0925) 65000, Ext. 239 Dr. I. H. Munro or Dr. D. Norman (Ext. 236). Application forms may be obtained from and should be returned, quoting reference number DL/773 to: The Personnel Officer, Daresbury Laboratory, Science & Engineering Research Council, Daresbury. Warrington, WA4 4ĂD. (9849)A

# THE WEIZMANN INSTITUTE OF SCIENCE is pleased to announce the availability of the Charles H. Revson Career Development Chairs

the Revson Chairs are intended for promising scientists who are eligible for appointment as senior scientists at the Weizmann Institute.

The Revson Chairs are intended for either scientists who reside in Israel or for those who are considering making their residence in that country.

The appointment for Revson Career Development Chairs are for three years. Appointees found suitable at the end of this period will be invited to continue their employment as permanent members of the Weizmann Institute's scientific staff.

Application forms and further information are available from: The Charles H. Revson Foundation Program, c/o The Academic Secretary, Weizmann Institute of Science, Rehovot 76100, (WARE)A Israel.

# **BIOLOGIST**

Expansion of the research programme has created an opportunity in our Agricultural Biology Department for a Research Assistant to join a team investigating the mechanisms of animal growth.

The job involves setting up laboratory studies using farm animals and monitoring their progress using radio-ligand assay techniques, interpretation of results and development of new assay methods.

Applications are invited from suitably qualified graudates/HNC's and those with directly relevant experience.

Our laboratories are located at Sandwich on the Kent Coast, where some 500 scientists and supporting staff are engaged in the discovery and development of novel compounds for use in human and veterinary medicine.

The starting salary will be competitive and will reflect qualifications and experience. There is good bonus potential.

Pension, death benefit schemes are in operation and there is assistance with relocation expenses. Flexible hours are worked and the social/recreational facilities are first-class.

Please write, in confidence, giving details of qualifications, experience, etc, to: G. Marshall, Personnel Officer, Pfizer Central Research, Sandwich, Kent. (9852)A



# FACULTY POSITIONS IN MOLECULAR GENETICS

The Department of Microbiology at the University of Cincinnati College of Medicine is undergoing a major expansion and invites applications for tenuretrack positions at either the junior or senior faculty level. Candidates should be involved in a vigorous and creative research program and should have demonstrated independence as laboratory investigators. Also the applicant should be an effective teacher and be able to direct graduate students. Research interests can be either in prokaryotic or eukaryotic molecular genetics. Applicants should submit curriculum vitae, a short statement of research interests and accomplishments, and the names of three scientists willing to write letters of recommendation. These should be forwarded to: Dr Jerry B Lingrel, Chairman, Department of Microbiology, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267. The University is an Equal Opportunity/ Affirmative Action Employer.

(NW027)A

# LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

(University of London)
Keppel Street/Gower Street,
London WC1E 7HT
DEPARTMENT OF MEDICAL
MICROBILOGY

(Head of Department: Professor A J Zuckerman) 2 MEDICAL LABORATORY SCIENTIFIC OFFICERS

1) for work in the Virology Unit and the WHO Collaboratoring Centre for Reference and Research on Viral Hepatitis. The emphasis of the work will be on tissue culture, immunochemical and structural analysis of viruses. The appointment will be initially for up to 3 years and prior practical experience in virus work is essential.

2) for work with and cultivation of chlamydia. The appointment will be initially for one year.

Salary in the range: £4,958 — £5,810 plus £859 London Weighting according to experience.

Apply in writing giving full details of qualifications and experience and naming two referees to the Assistant Secretary by 19th November 1981. (9834)A

# UNIVERSITY OF THE WITWATERSRAND

Johannesburg

# DEPARTMENT OF BIOCHEMISTRY LECTURER

Applications are invited from suitably qualified persons, regardless of sex, religion, race, colour or national origin, for appointment to the above post. Applicants are expected to have a PhD or equivalent and appreciable research experience.

The salary range is as follows: Lecturer: R10,995 — R19,230 pa (£1 = R1,75 approx).

The initial notch and level of appointment will be determined in accordance with the qualifications and experience of the successful applicant.

Intending applicants should obtain the information sheet relating to this post from The South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar (Staffing), University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001, South Africa, with whom Applications should be lodged not later than 30 November 1981. (9826)A

### POSTDOCTORAL POSITION

available for Cell Biologist with strong background in biochemistry to investigate the structure and function of organelles involved in cell wall formation by plant cells and protoplasts. Some knowledge of plant tissue culture and/or electron microscopy is desirable. The position is available immediately for a one year term (renewable for a second year) at a salary of \$15,000.00 to \$18,000.00 depending on experience and qualifications. Some travel assistance is available.

Interested candidates should send curriculum vitae and 2 letters of recommendation to Dr L C Fowke, Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0WO.

(NW026)A

### UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PHYSIOLOGY Faculty of Medicine and Dentistry

Applications are invited for the post of

# RESEARCH ASSOCIATE IB

to take part in a study of cardiovascular reflex responses in the systemic circulation and microcirculation. Applicants should have a good honours degree in physiology or related subjects.

Salary on the scale £5,285 to £7,700 with superannuation. Maximum starting salary £6,070. Post supported by the Medical Research Council for three years.

Further information from Dr J M Marshall, Physiology, 021-472 1301 ext 3458.

Applications (three copies) to Assistant Registrar, The Medical School, Birmingham B15 2TJ by 27th November 1981. Quote ref no RA/Phys/781. (9845)A

### FACULTY RESEARCH POSITION IN BIOCHEMISTRY

available at level of

### ASSISTANT PROFESSOR

Work involves biochemical studies obone regulation. Experience desir able in protein purification, cell cycl analysis, cell differentiation, immunochemistry, RIA membrane receptor studies, molecular genetics, o Vitamin D metabolism. Competitiv salary and exceptional opportunity is a well equipped, multidisciplinar laboratory.

Contact: D J Baylink, MD Department of Medicine, Roor 1501, Loma Linda University, Lom Linda, CA. 92350. Telephone 714/796-7311, extension 2171. A: Equal Opportunity/Affirmativ Action Employer. (NW024)A

### GEOPHYSICAL FLUID DYNAMICIST/PHYSICAL OCEANOGRAPHER

Applications are solicited for a junior faculty position in ocean physics or dynamics to begin in the academic year 1982-83. Areas of interest to the Department include analytical numerical and laboratory modeling of physical processes and phenomena in the sea.

Yale University is an equal opportunity/affirmative action employed and encourages women and members of minority groups to compete for this position.

Curriculum vitae, publications and the names of three or more referees should be sent by 3, December 1981 to: Robert B Gordon Chairman, Department of Geology and Geophysics, PO Box 6666, New Haven, CT06511. (NW1009)A

# EXPERIMENTAL POPULATION GENETICIST

with strong interest in eucaryotic ecology and evolution. PhI required. This is a tenure traciposition as Assistant/Associate Professor in Biology. Candidates will be evaluated for their potential to develop an outstanding research program with grant support and to interact with existing faculty in the genetics and ecology programs. Teaching will include upper and lower level courses in the area o specialty.

Application deadline: January 1 1982. Send CV, including reprints and three letters of reference to Chairperson, Search Committee Department of Biology, Box F, 20 Mueller Building, The Pennsylvanis State University, University Park PA 16802. An Equal Opportunity. Affirmative Action Employer.

(NW039)A

# **Experimental Therapeutics**

A new and progressive research group is to be formed within the Biology Department of G. D. Searle to study the Pharmacokinetics and therapeutic applications of new biological and chemical compounds. The establishment of this group complements the recent expansion of our Molecular Genetics and Chemistry Departments and is intended to provide information on the *in vivo* pharmacology of genetically engineered polypeptides and the metabolism of new chemical compounds at an early stage in their evaluation.

We are presently recruiting scientific staff for the three positions described below. Further scientific and technical vacancies are likely to become available as the work of the group expands.

# **Group Leader**

We seek an experienced and innovative scientist to head this new group. He or she will be a PhD with a first degree in a biological science or veterinary medicine and several years of post-doctoral experience in experimental pharmacokinetics or metabolism. The Group Leader will be responsible for establishing the group activities, maintaining high standards of research and creatively exploiting the potential for collaboration with other research groups. (Ref: B29)

**Biologist** 

An immediately post-doctoral scientist is required to assist the Group Leader with laboratory animal experimentation. Several years experience of *in vivo* pharmacology or experimental

metabolic studies would be advantageous. The successful candidate will be responsible for the design and execution of pharmacokinetic studies and will be assisted directly by highly trained animal technicians. (Ref: B41)

# **Biochemist**

A graduate in biochemistry is required to develop and apply chemical and biological assays to the study of natural and exogenous compounds in tissue fluids. His work will complement that of other analytical groups within the company and he will therefore primarily be concerned with the application of existing methodology to experimental work. Previous experience of automated techniques, HPLC analysis or clinical chemistry would be an advantage. (Ref: B42)

All the above positions carry competitive salaries commensurate with age and experience, and the Company offers 4 weeks' holiday a year, contributory pension fund, private medical scheme, subsidised cafeteria, and sports and social club.

Applicants, male or female, are invited to send a detailed CV or telephone for an application form from Miss D Wardman, Assistant Personnel Officer, Searle Research & Development, Lane End Road, High Wycombe, Bucks.

(Tel: High Wycombe 21124 ext 3374). Please quote appropriate reference.

(9829)A



### UNIVERSITY OF THE WEST INDIES Trinidad

Applications are invited for the post of RESEARCH

RESEARCH FELLOW/JUNIOR RESEARCH FELLOW

IN THE SEISMIC RESEARCH UNIT

Candidates should be well qualified in Geophysics, Geology or a related field. A doctorate and experience in operating seismograph networks is desirable. Salary scales (under review): Research Fellow TT\$29,784 — TT\$43,752 pa. Junior Research Fellow TT\$24,156 — TT\$26,484 pa. (£1 sterling = TT\$4.27). FSSU. Unfurnished accommodation or housing allowance. Family passages. Study and Travel Grant.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent as soon as possible to the Secretary, University of the West Indies, St Augustine, Trinidad. Applicants resident in UK should also send 1 copy to the Committee for International Copperation in Higher Education, The British Council, Higher Education Division, 90/91 Tottenham Court Road, London W1P 0DT. Further details are available from either address. (9824)A

# THE INSTITUTE FOR ADVANCED STUDY

will have several openings for members in theoretical physics and astrophysics for the academic year 1982-83. The positions are at a post-doctoral or higher level and applicants will be selected on the basis of their ability to do research in the areas of elementary particles, mathematical physics, astro-physics, plasma physics, general relativity and statistical mechanics. Preference is given to candidates who have received their PhD within the last year or two.

Postdoctoral members frequently collaborate with each other, with faculty members at the Institute or Princeton University, and with researchers at other institutions.

Appointments ae usually for no more than two years and support is typically full salary for postdoctorals and half salary for more senior persons. Women and minorities are encouraged to apply.

For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW1006)A UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH WELSH PLANT BREEDING STATION

Aplications are invited for the post of

# SCIENTIFIC OFFICER (I) HIGHER SCIENTIFIC OFFICER (I)

in the Station's Grassland Agronomy Department. This is a new post created under the Welsh Hills and Uplands R&D programme. The successful candidate will join the Department's exiting Hills and Uplands team but will carry out most of the field work at Pwllpeiran Experimental Husbandry Farm.

Candidates should possess a First or upper Second Class honours degree in agricultural, biological or soil science with at least two years relevant post graduate experience for appointment as HSO. Preference will be given to candidates with a PhD and a Knowledge of hill farming conditions.

Pay Scales: (S0) £5,176 - £6,964 (HSO) £6,530 - £8,589. Starting Salary according to qualifications and experience. Noncontributory pension scheme but male employees aged 18 or over contribute 1 ½ % to Widows and Orphans Fund.

Further particulars and application forms are available from The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed. Closing Date for applications is 27 November 1981. (Quote reference 68).

(9846)A

# Athens based

# **Technical** Manager

# Plant Products - Middle East

Cyanamid Overseas Corporation, part of the worldwide American Cyanamid Company specializing in the manufacture and distribution of a diverse range of pharmaceutical, chemical and agricultural products, wish to appoint an experienced Technical Manager for Plant Products in the Middle East.

Based in Athens, Greece, the successful candidate will be a technically qualified agricultural/plant products specialist capable of handling R and D work throughout the Middle East. This will necessitate extensive travel within the region.

Applicants should possess a M.Sc. in Agriculture (Entomology, Botany), have at least three years' practical experience in R and D work (insecticides/herbicides) and be familiar with the Middle East.

Please write in total confidence, with full c.v. and details of present income, to:

Personnel Department (C.O.C.), Cyanamid of Great Britain Limited, Fareham Road, Gosport, Hants, PO13 0AS.

Complete c.v.'s will be acknowledged and forwarded direct to our Athens office.



BSN-GERVAIS DANONE - France's no 1 food company with a rapidly increasing research programme - is seeking a

# molecular geneticist (PH.D)

preferably with experience in the microbiology of fermentation processes. A good working knowledge of the french language is necessary.

The successful candidate would hold a responsible position in a highly qualified team of 10 research scientists working in one of our laboratories, near

Applications accompanied by a curriculum vitae and a list of publications should be sent to BSN-Gervais Danone, Service Recrutement Cadres 7, rue de Téhéran 75008 PARIS (France) quoting reference D01N clearly on the envelope.

bsn.gervais danone bsn.gervais danone.

# BIOTECHNOLOGY

CHIRON CORPORATION, a young, rapidly expanding biotechnology company in the San Francisco Bay area, is inviting scientists with significant records of achievement in molecular biology and leadership potential, to join a strong team of innovative scientists in the application of recombinant DNA technology to the medical field.

We offer an attractive working environment close to the major universities of Northern California, competitive salaries, stock ownerships and an outstanding scientific advisory board.

If you are interested in becoming a partner in a young research company run by scientists, send curriculum vitae and references in strict confidence, to CHIRON CORPORATION. 2000 Center St., Suite 1212, Berkeley, California 94704.

### UNIVERSITY OF BIRMINGHAM FACULTY OF SCIENCE AND **ENGINEERING**

### **CHAIR OF MATHEMATICS** AND HEADSHIP OF THE **DEPARTMENT OF MATHEMATICS**

Applications are invited for the Chair of Mathematics and Headship of the Department of Mathematics. Appointment from a date to be arranged

Salary in the professorial range, plus superannuation.

Further particulars available from the Registrar, University of Birmingham, PO Box 363, Birming-ham B15 2TT, to whom applications (12 copies: 1 from overseas applicants) should be sent by 14 December 1981 (9828)A

# ASSISTANT PROFESSOR

The Department of Biological Chemistry of the University of Illinois invites applications from candidates for the position of Assistant Professor beginning in the academic year 1982-83. the candidate is expected to develop a research program in any area of biochemistry that will complement the activities of the existing faculty. Candidates should possess a PhD degree with a strong chemical background, at least two years of postdoctoral experience, and published evidence of ability to design productive experiments their intended area of biochemical research. Curriculum vitae and three supporting letters of recommendation should be sent to: Biological Chemistry Search Committee; University of Illinois Medical Center; 1853 W Polk Street; Chicago, Illinois

The University of Illinois is an Affirmative Equal Opportunity — Affirmative Action Employer and encourages women and (NW037)A applications from

# **UNIVERSITY OF EDINBURGH RESEARCH ASSOCIATE 1B**

Required for the immunoassay section of the department of clinica chemistry, 12 Bristo Place, Edin burgh. The person appointed wil undertake analysis of tumour marker in blood by radioimmunoassay, and assist in developing alternative methods for their analysis using monoclonal antibodies.

Applicants should be under 24 year of age and hold a degree in bio chemistry, or a related subject. The appointment is for a duration of two years, with a starting salary of £5,285 increasing to £5,675 in the second year. Arrangements to visit the laboratory can be made by contacting Dr J Seth at the above addres (031-229 2477 ext 2780).

Applications should be sent to th Secretary to the University, Universit of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, from whom further particulars may also b obtained. Closing date for applications, 20th November 1981. Pleas quote reference 5062.

(9842)A

# MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH LABORATORY OF BIOLOGICAL ULTRASTRUCTURE

A postdoctoral scientist with ex perience in biochemistry or neuro biology is required to join a group working on cytoskeletal elements in synaptosomes in relation to trans mitter mechanisms.

The appointment would be fo three years in the first instance in the salary range £7,290 — £8,92! plus £967 per annum London allow

Applications should be sent to the Director, National Institute fo Medical Research, Mill Hill, London NW7 1AA by 27th November 198 quoting reference BU/3RL

# **Medical Research Council Laboratories** Carshalton, Surrey

**Developmental and Cell Biology** 

Applications are invited for two SHORT-TERM NON-CLINICAL SCIENTIFIC posts tenable for 3 to 5 years according to age and experience of the successful candidate. Candidates with less than 3 years postgraduate experience will be considered.

Successful candidates will be required to conduct research in selected areas of mammalian embryogenesis. Experience in developmental genetics, cell and embryo culture, micromanipulation techniques will be welcomed.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff, plus London Weighting

Further information may be obtained from Dr. D. G. Whittingham, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, tel: 01-643 8000 ext 281, with whom applications — including a full CV and the names of two professional referees - should be lodged by 30th November 1981.



### THE UNIVERSITY OF LEEDS

PROCTER DEPARTMENT OF FOOD SCIENCE Applications are invited for a post of PRE-DOCTORAL RESEARCH ASSISTANT

n the above Department for work on he nutritional quality of fast soods/take-away foods involving the use of chemical and microbiological assay techniques. The post is available immediately for a fixed term of up to two years.

A good honours degree in Food Science or related subject is required. The post is suitable for a recent

Initial salary up to £6,070 on the IB Range for Research and Analogous Staff.

Informal enquiries may be made to Mrs J Ryley, Department of Food Science (tel. 0532 31751 ext 548).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 JT, quoting reference number 72/9 Closing date for applications 19 November 1981. (9851)A

### HIGH ALTITUDE **OBSERVATORY**

Visitor Appointments at the High Altitude Observatory are available for new and established PhD's for up to one year periods to carry out research in solar physics, solar-terrestrial physics, and related subjects. Applicants should provide a curriculum vitae including education, work experience, publica-tions, the names of three scientists familiar with their work, and a statement of their research plans. Applications must be received by 15 January 1982, and they should be sent to: Visitor Committee, High be sent to: Visitor Committee, Fig. Altitude Observatory, National Center for Atmospheric Research (NCAR), PO Box 3000, Boulder, Colorado 80307, NCAR is an Equal Opportunity/Affirmative Action Employer. (NW931)A

### **WASHINGTON UNIVERSITY**

SCHOOL OF MEDICINE DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

# **POSTDOCTORAL POSITION**

is available immediately to study gene expression and recombination in RNA viruses of eucaryotes. Research emphasizes the molecular biology of defective interfering virus particles and their role in virus persistence.

Studies include cloning and sequencing of viral genome deletions and regulation of viral polymerase activities. Salary is equivalent to NIH

Send applications, including curriculum vitae and names of three references, to Dr Jacques Perrault, Box 8093, Department of Microbiology and Immunology, Washington University School of Medicine, St Louis, Missouri 63110 USA. Equal Opportunity/Affirmative Action Employer M/F/H. (NW020)A

# YALE UNIVERSITY DEPARTMENT OF GEOLOGY AND GEOPHYSICS

Applications are solicited for a faculty position in solid earth geophysics to begin in the academic year 1982-83. Areas of interest to the Department include seismology, exploration geophysics, mechanical and physical properties of rocks and minerals, geomagnetism, and tectonophysics.

Yale University is an equal opportunity/affirmative action employer and encourages women and members of minority groups to compete for this position.

Curriculum vitae, publications and the names of three or more referees should be sent by 31 December 1981 to Robert B Gordon, Chairman, Department of Geology and Geophysics, PO Box 6666, New Haven CT 06511. (NW1010)A (NW1010)A

# Medicinal **Chemist**

Pfizer Central Research is part of a multi-national organisation engaged in the research, development, manufacture and marketing of a wide range of human medicinal and animal health products. Located at Sandwich, on the Kent Coast, are our modern, well equipped facilities where about 500 scientists and supporting staff are engaged in the discovery and development of novel compounds for use in human and veterinary medicines.

Recent expansion of our project portfolio has created an opportunity for an innovative Medicinal Chemist, with 3-5 years' post-doctoral experience of research in the pharmaceutical industry, to lead a small team of chemists on a new project to discover substances which reverse certain aspects of microbial pathogenicity. He/she will collaborate closely with a recently established team of biologists already working in this area.

The person we seek will have originality, drive and a desire to remain in basic research for many years. There is ample opportunity for the successful candidate to gain promotion to positions of higher scientific management.

The person matching our needs can expect a very competitive starting salary with good bonus potential. Pension and death benefit schemes are in operation and there is generous assistance with relocation

expenses. Flexible hours are worked and the social/recreational facilities are first-class.



Applications, giving details of qualifications, age, experience, etc., will be treated in strictest confidence and should be sent to: D. W. Sells, Personnel Manager, Pfizer Central Research, Sandwich, Kent.

(9818)A





# **Royal Postgraduate Medical School**

(University of London)

Department of Clinical Pharmacology in collaboration with Imperial Chemicals Industries Limited Monoclonal Antibodies to Cytochrome(s) P-450

A graduate with a good honours degree or equivalent experience, in biochemistry or pharmacology is required to join a Joint Research Scheme project between the Department of Clinical Pharmacology, Royal Postgraduate Medical School and Imperial Chemical Industries Limited (Central Toxicology Laboratory and Corporate Biosciences Group). The aim of the project is to produce monoclonal antibodies to cytochrome(s) P-450 from human and rodent liver. These antibodies will be used for the characterisation of cytochromes and in their purification. The successful applicant will be involved in helping in the characterisation of the antibodies with respect to their reactions with the cytochromes P-450.

The person appointed will join an existing team at the Royal Postgraduate Medical School consisting of analytical chemists, pharmacologists and an immunologist. The appointment is for 2 years with a starting salary between £5,451 and £6,556 depending on

Application forms and further details are available from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS, quoting reference number 20/211.

Closing date for applications 20 November 1981.

# Senior Pathologist

# Pharmaceutical Research

This is a senior and important appointment within the expanding UK Research Centre of a well-known international pharmaceutical company

Reporting to the Director of Toxicology and Pathology, the successful candidate will be responsible for the identification, interpretation and evaluation of morphological and pathological changes in animal tissues, as part of the safety evaluation of new

Ideally candidates should be veterinary pathologists, but the appointment could be open to well-experienced comparative animal pathologists who do not possess formal academic qualifi-cations. The company is prepared to structure the post according to the background interests and potential of the person appointed for someone whose interests and experience to date are primarily scientific, it could be as a specialist histopathologist; for someone with appropriate qualifications, experience and management skills, it could be as Head of Pathology - leading a small, well experienced multidisciplinary team.

A highly competitive salary will be negotiable individually, according to qualifications and experience. Other benefits include non-contributory life assurance and pension schemes, and generous relocation expenses if required

Please write in confidence for an application form, or phone if you would like to discuss the appointment further before applying.



Talentmark
Leaders in Health Care Recruitment

James A Edwards, Talentmark Limited, King House, 5-11 Westbourne Grove, London W24UA. Telephone: 01-229 2266.

(9844)A

### THE DISTILLERS **COMPANY LIMITED ASSISTANT SCIENTIST**

The Food Group of the Distillers Company Limited require an Assistant Scientist for work in the Spirit Yeast Fermentation Laboratory of their Yeast Research Department, Menstrie, Clackmannanshire.

Applicants should have a HNC qualification in chemistry, preferably with 2 or 3 years laboratory ex-perience in the use of fermentation or biochemical techniques and including the handling of gas liquid chromatography equipment. Applications from candidates possessing qualifications other than HNC will not be considered further. The person appointed would be directly responsible to the Microbial Biochemist in charge and would assist him in research projects as well as in routine laboratory work with eventual responsibility for day to-day laboratory operations.

An application form can be obtained by writing to: The Yeast Research Manager,

The Distillers Company Limited, Food Group,

Research Department, MENSTRIE.

Clackmannanshire FK11 7ES. Closing date for applications November 20th, 1981. (9816) (9816)A

# LINIVERSITY OF SASKATCHEWAN

DEPARTMENT OF **GEOLOGICAL SCIENCES** 

Applications are invited for a vacant tenurable position in

# **ENGINEERING** GEOLOGY/GEOPHYSICS

Applicants should be qualified to teach undergraduate and graduate courses and to conduct research in engineering geology. A background in structural geology may be appropriate. Well-equipped facilities are available for research in rock mechanics, fluid flow through porous media, acoustic and electrical properties of rocks, and permafrost. Rank and salary commensurate with qualifications and experience.

Send applications, detailed personal résumé including the names of at least three referees, and other supporting data to Dr W G E Caldwell, Head, Department of Geological Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0WO.

Please note: until November 15, 1981 consideration will be given only to applicants who are Canadians or landed immigrants, after that date all applications will be considered.

(NW033)A

# **UCLA SCHOOL OF MEDICINE** DEPARTMENT OF MICROBIOLOGY AND **IMMUNOLOGY**

Applications are solicited for a tenure track faculty position in the area of

VIRAL IMMUNOLOGY

at the rank of Assistant Professor Outstanding applicants possessing a doctoral degree, postdoctoral experience and a strong commitment to developing an independent research program are sought. Applicant must be inte-rested in studying host response to viral diseases using immunological approaches. Applicant must be well trained in both conceptual and technological aspects of modern immunology, virology and possibly molecular biology and must show a strong promise of independent research capability. Responsibilities include participation in the departmental teaching of medical, dental and/or graduate students as well as possibly developing a graduate course in viral immunology. Please submit curriculum vitae, current research interest, list of publications and names of references. Nominations of outstanding candidates are also

Debi P. Nayak, BVSc, PhD Professor and

Jack G. Stevens, DVM, PhD Professor and Chairman Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024. UCLA is an equal opportunity and affirmative action employer.
(NW044)A

### **UNIVERSITY OF** LIVERPOOL CHAIR OF SMALL ANIMAL STUDIES

Applications are invited for the newly established Chair of Small Animal Studies. The person appointed will be expected to encourage academic staff of the Small Animal Hospital actively to pursue specialist clinical interests and research in addition to clinical teaching duties. It would be an advantage if the successful applicant were an authority in a branch of small animal internal medicine but other disciplines are not excluded.

The salary will be not less than £16,480 per annum.

Applications (12 copies) together with the names of three referees, should be received not later than 11th December, 1981, by the Registrar, The University, PO Box 147, Liver-pool L69 3BX, from whom further particulars may be obtained. (Candidates overseas who wish to do so, may send only one copy by airmail). Quote ref RV/899/N.

(9819)A

Please mention

# nature

when replying to these advertisements

### **TEACHING POSITION**

The Department of Geology, Carleton University, invites applications for tenure-track faculty position at th Assistant or Associate Professor level Appointment date: July 1, 1982. Car didates must have a PhD degree, witl a strong interest in field research. Mus be able to teach graduate and undergraduate courses in at least on of the three principal fields of research at Carleton: Resource Geology Structure and Geodynamics, and Precambrian Geology. As required by Employment and Immigration Canada regulations, only Canadian or Landed Immigrants to Canadaneed apply for this position.

Send curriculum vitae and the names of three referees, beford January 15, 1982, to: Dr J / Donaldson, Chairman, Departmen of Geology, Carleton University Ottawa, Ontario K1S 5B6. This position is subject to budgetary approval. (NW038)A

# UNIVERSITY OF **BIRMINGHAM** DEPARTMENT OF GENETICS POST-DOCTORAL RESEARCH FELLOW

Applications invited for this ARC funded post tenable from 1 February 1982 until 31 October, 1984 to worl on intra and inter specific gen transfer in Nicotiana species unde the supervision of Professor J L Jinks

Salary on Research Fellow 1A scal £6,070 — £10,575 plus super annuation. Maximum starting salar

Further particulars from Assistan Registrar, (Sci & Eng), PO Box 363 Birmingham B15 2TT to whom ap plications (three copies) including fu curriculum vitae and naming thre referees should be sent by 2 November, 1981.

Please quote ref: NL1. (9831)A

# PRINCETON UNIVERSITY ASSISTANT PROFESSOR

We seek a geologist trained in Sedi mentology and/or Paleontology witl research interests in Phanerozoic o Precambrian sedimentary geolog and history. Appointment is to b effective September 1982 or late depending on availability o candidate.

Applicants should send résumé to: Sheldon Judson, Chairman Department of Geological and Gec physical Sciences, Princeton University, Princeton, New Jersey 08544.

For further information: Rober Hargraves, Chairman, Search Com mittee, Department of Geologica and Geophysical Sciences, Princeto University, Princeton, New Jerse

Princeton University is an equa opportunity affirmative actio employer. (NW036)A employer.

# UNIVERSITY OF THE WITWATERSRAND

Johannesburg

DEPARTMENT OF GEOLOGY

### CHAIR OF GEOCHEMISTRY

\*applications are invited from suitably qualified persons, regardless of ex, race, colour or national origin or appointment to the above post.

The successful applicant will be esponsible for the co-ordination and advancement of teaching courses in geochemistry, and in addition to bursuing his/her own research projects, will be encouraged to participate in a wide range of interfisciplinary Earth Science projects. The Department is well equipped with an experimental geochemical aboratory and XRF, XRD, wethemical analytical facilities, and a modern ARL-SEMQ electron microstrobe which is operated and maintained by an electron microprobe with an experimental analyticated neutron and charged-particle activation actilities with an associated adiochemical laboratory, as well as table and natural radioactive isotope analytical facilities.

The salary will be determined according to qualifications and experience in the range R20,040 — R26,250 per annum. (£1 = R1.75 approx).

Intending applicants should obtain he information sheet relating to this bost from the Director, South African Universities Office, Chichester House, 278 High Holborn, London WCIV 7HE, or rom the Director: Personnel Office, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa 2001, with whom applications hould be lodged not later than 30 November 1981. (9823)A

FWO Chemistry tenure-track faculty positions beginning September 1982. Rank and speciality open. Excellence in research and teaching required. Send résumé and research plans and nave three letters of recommendation ent to N N Lichtin, Chairman, Dept of Chemistry, Boston University, Boston, MA 02215, by January 31, 981. Affirmative Action/Equal Deportunity Employer. (NW041)A

### LA TROBE UNIVERSITY Melbourne, Australia

SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF GENETICS AND HUMAN VARIATION

### LECTURER (TENURABLE)

A position is available for early 1982 for a lecturer with research experience in molecular aspects of eukaryote gene structure and expression. Applicants should have a strong training in general genetics as some undergraduate teaching in prokaryote genetics will be required. The appointee will be expected to be actively involved in research including the supervision of honours and postgraduate students.

The facilities of the Department include glasshouse, growth cabinets, constant temperature rooms, a CI containment room, analytical and other centrifuges as well as routine items of potential assistance for a molecular geneticist.

Applicants for the previously advertised fixed-term appointment will be considered and need not reapply.

Salary: \$A19,821 - \$A26,037.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications marked confidential and quoting reference number 150/32/22 close with the Staff Officer, La Trobe University, Bundoora, Victoria, Australia, 3083 by 11 December 1981.

# TRANSLATOR — FISHERIES AQUATIC BIOLOGY

Russian to English. Excellent command of English, Russian, and subject matter required. Scripta Publishing Co, 7961 Eastern Ave, Silver Spring, MD 20910.

(NW025)A

# SEMINARS and SYMPOSIA

# **SYMPOSIUM**

# "BIOMEDICAL RESEARCH: IMPORTANCE OF LABORATORY ANIMAL GENETICS, HEALTH, AND ENVIRONMENT"

The Fifth Charles River International Symposium will be held at the Sheraton Airport Frankfurt, Frankfurt, West Germany on March 9 & 10, 1982. Sponsored by the Charles River Foundation, the symposium will examine the importance of the laboratory animal to research and the researcher. For program and registration information:

Deutsches Reiseburo Der Congress Eschersheimer Landstr. 25-27 6000 Frankfurt Am Main West Germany Attention: Mrs. Rockmann

(NW029)M

**CONFERENCES and COURSES** 



November 16-17, 1981 Hilton International Düsseldorf Düsseldorf, West Germany Nov 30-Dec 1, 1981 The Drake Chicago, Illinois



# ROBERT S. FIRST, INC.

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(NW014)C

# AWARDS

# Nominations are invited for the 1982 Rosenstiel Award in Oceanographic Science

This award, which is administered on behalf of the Rosenstiel School of Marine and Atmospheric Science (RSMAS), University of Miami, recognizes outstanding contributions to marine science, including oceanographically relevant aspects of atmospheric science, and fundamental developments in ocean engineering. The award consists of a cash prize, currently \$5,000, and a medal. The recipient of the award will be invited to spend a week at RSMAS for discussions with faculty and students. The award will be presented at a banquet at that time.

To accommodate the multidisciplinary value of oceanographic science, the award recognizes, on a rotating basis, achievements in four broad disciplinary areas. In 1982 the emphasized discipline will be marine geology and geophysics. The achievements recognized may consist of contributions towards the development of ocean science in general, or of more focussed-individual research or recognized impact on our understanding of the marine environment.

Nominations for the 1982 award for outstanding achievement in marine geology and geophysics should be directed to the Interium Dean, Warren J. Wisby, Rosensteil School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149, before March 15, 1982. Nominations should include a brief justification together with relevant references, and a c.v. if possible. The selection panel would especially welcome nominations of outstanding younger scientists whose early contributions suggest a continued role of leadership in the field.

Previous recipients of the award in Marine Geology and Geophysics have been Edward Ringwood, Kenneth Emery, and John Sclater.

# **FELLOWSHIPS**

The Max-Planck-Institute for Psychiatry, Department of Neurochemistry, in Munich, Federal Republic of Germany, offers a

POSTDOCTORAL RESEARCH FELLOWSHIP for Research in Molecular Neurobiology

Applications are invited for a 2-5 year postdoctoral appointment to begin as early as January 1982. Research involves the isolation of neuron-specific cDNA probes as tools for the study of nervous system development. Experience with DNA sequencing, recombinant DNA techniques and DNA-RNA hybridization analysis is essential. Approximately salary is 40,000 to 50,000 DM.

Applications should be sent to Dr. Louis De Gennaro, Yale University School of Medicine, Dept. of Pharmacology, P.O. Box 3333, 333 Cedar St., New Haven CT 06510, USA, or to Dr. H. Thoenen, Max-Planck-Institute for Psychiatry, 8033
Martinsried, Federal Republic of Germany. (NW022)E

### **POSTDOCTORAL FELLOWSHIPS IN ZOOLOGY**

The Department of Zoology at the University of Florida announces one or two postdoctoral research positions starting August 1982. The duties of the appointments, (\$15,000/12 months, renewable for a second year), will be to establish an effective research project in comparative, ecological or evolutionary zoology. The Department will provide a list of faculty and their recent publications upon request. Candidates should submit the names of three referees, curriculum vitae, abstract of dissertation, and a proposal of their intended research, which should include a statement of the appropriateness of the University of Florida for the research. Direct in-

quiries and submit applications to:
Dr. Brian K. McNab Chairman, Postdoctoral Search Committee,
Department of Zoology, University of Florida, Gainesville FL

32611 904/392-1178.
Application deadline 1 February 1982.
An Equal Opportunity / Affirmative Action Employer.

(NW040)E

# **UNIVERSITY OF NEWCASTLE UPON TYNE FELLOWSHIPS**

Applications are invited for the following Fellowships, tenable for two years from 1 October 1982, from persons who have shown themselves able to carry out original research. The value of each Fellowship is £6,070 in the first year and £6,475 in the second year.

# SIR JAMES KNOTT **FELLOWSHIPS**

Two Fellowships available in any Faculty (normally awarded to graduates of other Universities) LORD ADAMS **FELLOWSHIP** 

One Fellowship available in any Faculty

preference will be given to candidates who, through family, residence or education, are connected with the former County of Cumberland or whose proposed research has connections with, or will be of benefit to that County.

Further particulars and application forms (which must be returned by 31 December 1981) may be obtained from the Registrar (Fellowships), University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU. (9833)E 7RII

# THE BRITISH COLUMBIA **TERRY FOX TRAINING CENTRE**

### announces initiation of a FELLOWSHIP PROGRAM

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Applications must be received by May 1st and December 1st of each year (starting December 1st, 1981), and should be sent with curriculum vitae and three references to: Allen C Eaves, MD, PhD, Co-Ordinator, Terry Fox Training Centre, BC Cancer Research Centre and Cancer Control Agency of BC, 601 West 10th Avenue, Vancouver, BC Canada Avenue, V5Z1L3. (NW023)E

## THOMAS C USHER RESEARCH FELLOWSHIPS AND STUDENTSHIPS TENABLE AT THE MRC LABORATORY OF **MOLECULAR BIOLOGY** IN CAMBRIDGE

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Applications should be addressed to Dr M F Perutz, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK and be accompanied by a curriculum vitae, the names of at least two referees, and for categories (1) and (2) only, a list of publications and a research proposal of between 300 and 1,000 words. Applications should reach Dr Perutz by 16th April 1982. (9825)E

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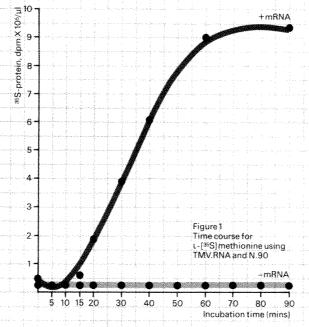
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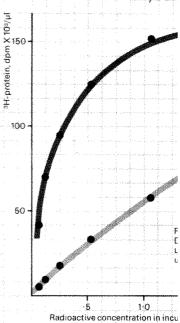
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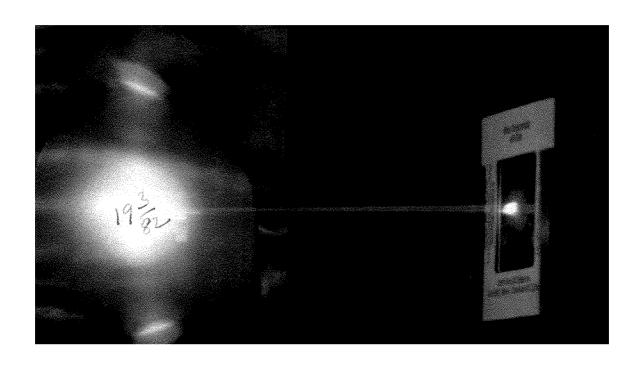
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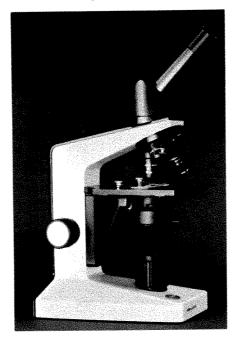
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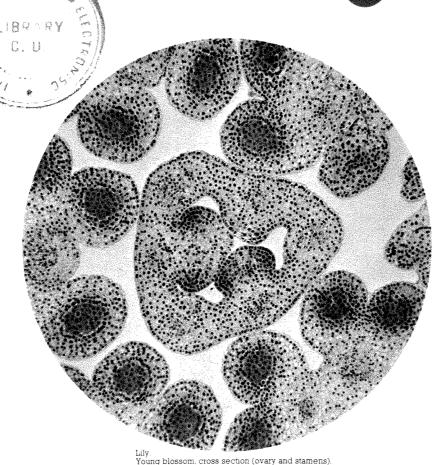
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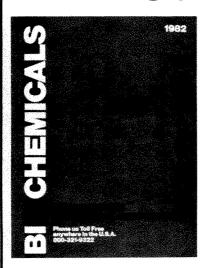
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# nature

12 November 1981

# What case for basic research?

Researchers threatened by the budgetary process in the United States are preaching the virtues of basic research. But even Dr Frank Press's argument will not wash. What should be done?

The budgetary process in the United States, always confused, is this year causing more than the usual havoc. There is now a serious danger that, whatever is decided, needless damage will be done to important scientific institutions. For although it is customary that the Administration's authority to conduct government business at the beginning of the financial year stems from a continuing and temporary resolution by Congress, the sequence of events this year will provide the new President's men with a licence to spread alarm and despondency among the agencies they control. Some, to their shame, seem to have leaped at the opportunity. But even when the heads of government agencies have been resolute and far-sighted, research managers have been forced to think what has hitherto been unthinkable. The general mood of alarm has spread widely through that part of the American research establishment supported directly by the Administration. Further afield, those who will be looking for research grants from the grant-making agencies are despondent or are making eyes at the Department of Defense, one of the few agencies of the United States government certain to have more to spend. Whatever damage is done by whatever budget is eventually agreed, the mere process of getting something settled is now certain to leave a scar on the research enterprise. Sooner rather than later, something must be done to put things right.

# **Budget**

Since the beginning of the year, Congress has been faced with no fewer than three versions of the budget for the year that started on 1 October, beginning with President Carter's valedictory budget which must in retrospect be read as his declaration of how well-heeled everybody would have been if only they had voted for him in sufficient numbers. The framework of the new budget appeared only in March, together with President Reagan's proposals for reductions in the rates of income and corporation taxes that, in what is almost certain to prove a pyrrhic victory, the President carried through Congress early in the summer. Left alone, Congress might well have lived up to form and enacted some kind of budget authority in the usual wild legislative scramble between Thanksgiving Day and Christmas. The trouble, which became apparent during August, is that the March budget coupled with the tax cuts would require the United States to run a deficit of between \$60,000 million and \$100,000 million in the twelve months to next October. Seeking to retain some semblance of Republican virtue in the management of its affairs, the Administration accordingly proposed in September a 12 per cent cut in the discretionary expenditure of all its agencies except the Department of Defense (whose increased budget was cut by \$3,000 million in August) and the National Aeronautics and Space Administration (whose spending is reduced by only 6 per

Congress is now trapped in a familiar dilemma. The Administration is right to argue that deficit financing will only sustain inflation and prolong the period when interest rates remain high, but agreeing to the cuts proposed will be unpopular with the voters (who will be going to the polls only a year from now). So members of Congress are cheerfully denying the President the 12 per cent cut he asks for, preferring that he rather than they should incur the odium of appearing mean, which is what will happen if he should veto the inflated appropriations bills. The only quick way out of the impasse that has been created

is that the Administration should agree to a postponement of its plans for the MX missile and the B1 bomber, against which both houses of Congress have raised cogent objections, in which case the inflationary implications of the present budget will seem less threatening. That, however, would be a climb-down for the Administration which is unlikely to be undertaken lightly or quickly. In the meantime, the agony in the research community will continue.

Rumours abound of what the consequences will be. Will it be necessary to close whole laboratories? The Brookhaven National Laboratory is potentially a casualty if its new accelerator is abandoned (see Nature 5 November, p.3). And if it turns out that the National Aeronautics and Space Administration has no funds left over from the ill-starred space shuttle, can it make sense to keep the Jet Propulsion Laboratory alive? Thus, almost casually, the fate of powerful components of the American research enterprise are loudly discussed. No wonder that those concerned are making discreet enquiries of the Pentagon about the military tasks they might take on. Not all establishments are as fortunate, however. The laboratories of the Bureau of Drugs whose members have been told their jobs are not of the highest priority (see page 101) will be kept on tenterhooks for many months to come. Elsewhere, even in laboratories whose usefulness is widely acclaimed, research directors are compelled by sheer prudence to draw up lists of people whose jobs may have to go on the insubstantial grounds that, with more than a tenth of the financial year already gone, and while the possibility remains that they may have to reduce expenditure by an eighth before the financial year is over, preparing to fire people soon is more important than the prosecution of an imaginative research programme. This is no way in which to deal with intellectual enterprises. So much, both Congress and the Administration must be given clearly to understand.

# Message

But how? What kind of message is likely to carry weight with an Administration apparently less sensitive than its predecessors to intellectual interests and a Congress preoccupied, as always, with the problems of its own reelection? Dr Frank Press's colloquium at the National Academy of Sciences two weeks ago (see Nature 5 November, p.4) was a first attempt at putting the case for basic research to the Administration, but is unlikely to carry much weight. For the plain truth is that in the United States, as in Britain for the past several years, basic research as such has come to be regarded as effete, a kind of luxury. Everybody knows that the pursuit of a deeper understanding of natural phenomena gives great pleasure to those directly engaged in it, while even the most cynical legislators are prepared to hail the award of a Nobel prize to a fellow-countryman as a mark of their own percipience (which is naturally magnified a hundredfold if the prizewinner should be a constituent). To do them justice, however, legislators are also properly preoccupied with the larger question of how they can be held by their constituents or anybody else to have behaved responsibly if they condone inflation and thus, by extension, the erosion of the value of property, the undermining of established institutions and the setting of a trend towards the fly-by-night. Dr Press's "consensus statement" made a seemly acknowledgement of the United States Administration's economic problems. It went disastrously awry in asking that if government support for

science and technology had to be reduced, basic research should be given priority over development and demonstration. Even the Majority Leader in the Senate, Senator Howard Baker, has helped in the past few days to breathe new life into the most spectacular demonstration of all—the technically antiquated fast reactor at Clinch River. It is, however, unlikely that Senator Baker did not know of Dr Press's colloquium. More probably, he did not care.

# Incorrigibility

So are the Senator Bakers of this world incorrigible? Or is it that those engaged creatively in basic research are indefinitely and repeatedly dispensable? These questions are not easily dealt with. Most politicians will accurately calculate that if the population of those now engaged on basic research is so eager to continue with that pursuit, the recruitment of a substitute should be relatively easy once it again becomes affordable. Moreover, the calculation is entirely correct. There will never be a shortage of people willing to deepen understanding if offered the chance. What is in question is their capacity to do so in a vacuum. The experience since the Second World War of a variety of nation-states selfconscious about their conduct of basic science has shown convincingly that success depends on the ease with which relatively young people can hit the ground running, as the appropriately American idiom has it. This in turn entails that the social function of senior and often distinguished researchers is to serve as a means by which young people can make a quick start. Newton acknowledged his debt to the giants on whose shoulders he stood; but the fabric of scholarship is such that even the shoulders of pygmies (relative to, say, Copernicus) are literally infinitely better than nothing. The first and the most important defence of basic research, in the United States and elsewhere, is that the process is cumulative. Not to have shoulders of any kind to stand on would spell some end-point for the research community which, for the whole of the past three decades, has been the chief source of intellectual innovation anywhere.

Unfortunately, the most immediate implications of this truth are that all basic research is equally valuable as a means of launching young and innovative people on the natural world. In present circumstances, that is probably as far from being the case in the United States as elsewhere. Indeed, the plurality of possible sources for the support of basic research of which the United States can still boast, and the freedom accorded by universities and other academic institutions to those who can recruit funds, ensures that even inward looking projects win an honourable place in the research enterprise. It would have been more persuasive, of Senator Baker but also of the United States Administration, if Dr Press's consensus document had squarely promised that the academic community would itself arrange that young people would always have durable shoulders on which to stand. Briefly, if the National Academy of Sciences wishes, as it should, to persuade the Administration to acknowledge the importance of continuity in basic science, it must also itself take on some responsibility for defining the circumstances in which basic research should be supported.

### Default

By default, the academy's consensus statement last week fell back on the weaker argument that industry would be robbed of the skilled people it needs if basic research is deprived of the funds to which it has grown accustomed. This line of argument is persuasive among academics. Unfortunately, neither the government nor industry, the supposed beneficiary, is likely to be impressed. Each of the sceptical but interested parties is fully aware that, for several years, the computer industry in the United States has been forced to manage without a sufficient supply of trained engineering graduates from the universities and that it has nevertheless managed creditably to look even Japanese competition in the face. Who would say that more support for basic science in the past decade would have remedied this state of affairs? And who would hold that academic institutions are necessarily the places at which the people best qualified to design

the next generation of integrated circuits, or the next elaboration of sophisticated software systems, should be trained? Most probably, even those who contributed to Dr Press's consensus document last week would judge this to be too pointed a question, too particular. The potential benefits of basic research, the riposte would go, by definition lie in fields that cannot as yet be defined. Yet Congress, the Administration and the throng of taxpayers and voters increasingly hag-ridden by inflation are understandably concerned with solving the unsolved problems that can be foreseen. Is it too much to ask that the basic research community should take time off from its usual preoccupation with the design of research-grant proposals to pay some attention to the preparation of young people for the modern world?

In such circumstances, the hope (still buoyant among even American academics) that industry will somehow step in to make good the deficiencies of federal spending is uncharacteristically unrealistic. Industry at present contributes only a small part of the cost of basic research carried out at universities and other research laboratories not directly controlled by government or by itself. For various reasons, not always calculations about the best way of paying less tax, corporations will no doubt seem more generous in the years ahead. (The public rhetoric will help.) Yet there is not the slightest hope that industry would be able — even if it were willing - to meet the full cost of reinvigorating engineering education in the United States. Moreover, there is the strongest reason why the basic research community, essentially the academic research community, should resist such a development — a university's chief responsibility is to its students and not to those who may eventually become its students' employers. It follows that the cost of university education, and of the research that keeps that education alive, should fall either on the students or on the community to which they belong. Third parties have no place. Paradoxically, then, the basic research community can best serve the needs of American industry by remaining as far as possible financially independent of it.

# Change

Thus it is that the basic research community is faced with a version of the dilemma with which Congress and the Administration are confronted. How, when the cost of carrying on without change is insupportable but when change as such is unacceptable, is the essence of an enterprise, or of an institution or even of a community (say, a nation-state) to be preserved intact? Congress is denying the Administration the budget cuts for which it asks because Congress cannot accept that its constituents' expectations of the modern world should be disappointed. The Administration is (so far, at least) inflexible on its defence budget because it dare not risk the frustration of its election promises. Both parties to this non-bargain know, however, that their failure to reach an accommodation is a recipe for disaster - one that would touch all sections of the community, not merely that concerned with basic research. The participants in Dr Press's colloquium might with advantage have taken hold of that point more directly. It is not merely their government's problem but theirs. Within their own parish, they should also have had the wit to recognize that among the chickens that have come home to roost are some born of academics' own firm belief that basic research is an occupational right (and that graduate students exist for the education of undergraduates); that the curriculum that has grown up in no more disciplined a way than Topsy is nevertheless sacrosanct; and that the time has come if not for change then for adaptation.

Fortunately, the American system of basic research is still so much more varied than any other that much of it is likely to survive the hard times ahead. The research community is right to protest at the wayward incidence of the latest round of budget cuts, and at the way in which they arbitrarily affect those projects that can most easily be cut. It would also be within its rights to complain that both the Administration and Congress have undervalued its contribution to the health, wealth and adaptability of the community in which it is embedded. But it cannot hope not to have change itself.

# Closure threat at FDA laboratories

# Drug testing facilities given low priority

Washington

The Food and Drug Administration (FDA) seems to be playing cat and mouse with the 500 members of the staff of its drug research laboratories in south-east and south-west Washington. Last month, as the Reagan Administration's budget problems became apparent, the FDA management raised the prospect that its three drug investigation laboratories might be shut. At the same time, all members of staff were advised that they should ensure that their career record forms (SF171) were complete by the end of October. This is a familiar preparation for dismissal. Although the Senate Appropriations Committee has now decided that the emergency cut of \$40 million in FDA's budget should be trimmed by \$6 million, members of staff have the impression that the laboratories' days are numbered.

FDA's response to last month's budgetary problems seems to have been unusually panicky. Senior officials of the administration were given 24 hours in which to define a package of economies amounting to 20 per cent of the budget. At a meeting on 16 October in the auditorium of FDA's Humphrey Building, more than 200 scientists were told that the investigation had shown that the Bureau of Drugs could dispense with its divisions of drug biology and of drug chemistry and the biopharmaceutics laboratory. It had already been decided that the national centre for antibiotic analysis, supported chiefly by fees from manufacturers with products to test, will be disbanded, largely on the grounds that its work could be carried out by private laboratories.

Anxiety about the future of the laboratories stems from the form of words used at the meeting on 16 October by Dr Richard Terselic, deputy director of the Bureau of Drugs. Terselic is reported to have told his audience that on several occasions in the past two or three years, studies of the consequences of large budget cuts had repeatedly shown the laboratories to be dispensable. Asked what would happen if the cut required of FDA was more like 5 per cent than 20 per cent, he is reported to have said that "if you have decided that A is less important than B. . . there is a pressure in the system" that will make the less important activity vulnerable in future years.

A spokesman for FDA confirmed last week that the continuation of the work of the laboratories "has been identified as a low-priority area". He added that in the light of the action by the Senate Appropriations Committee, it was not now likely that the laboratories would be closed during the present financial year (which began on 1 October).

FDA's drug investigation laboratories occupy a central role in its regulation of new drugs. Their functions include the checking of manufacturers' claims for the safety of new materials and an assessment of the efficacy of materials proposed for medicinal use. Among the laboratories' claims on public attention is that they were the source of the first warnings of the dangers of thalidomide in the late 1950s.

Members of the laboratories claim that

closure would compel the Bureau of Drugs to rely on post-marketing surveillance for the independent assessment of the efficacy of new drugs. It seems likely that such a departure from present practice would raise hackles in Congress. A letter-writing campaign by those concerned has been begun, while earlier this week it seemed likely that Congressman Albert Gore, chairman of the oversight subcommittee of the House of Representatives Committee on Science and Technology, would try to get to the bottom of FDA's intentions.

The outcome of a mandatory meeting of all the laboratory staff called for Tuesday this week is not yet apparent.

# UK universities plan to fire staff

British universities are still in a state of confusion over how to economize to meet the cuts in their grants. A major worry is still whether compensation to academics who lose their jobs will cost the universities more than savings in lost salaries. The answer will depend on how much extra money the government is willing to make available specifically for redundancy payments.

The Committee of Vice-Chancellors and Principals, which expects the first redundancies to be announced within a few weeks, now says that the matter is urgent. Last week it asked the University Grants Committee (UGC), which distributes government money among universities, to announce the terms under which it will compensate universities for redundancy payments.

The vice-chancellors' committee has provided the UGC with its own recommendations for the minimum compensation to which redundant academics should be entitled. Its scheme is intended to ensure fair treatment, especially for those academics whose contracts of employment do not give them security of tenure. Those with tenure, however, are likely to fight for higher compensation through the courts, making it still impossible to calculate the total redundancy bill. The best estimate remains that made in the summer of about £250 million.

The vice-chancellors' compensation scheme is based on that for civil servants. It is divided into three age categories — above 55 years, 50–55 years and below 50 years. Under the scheme, academics aged 55 years and over would be entitled to compensation broadly in line with the existing premature retirement scheme which provides a lump sum and an early pension. For example, an academic aged 55 years with 30 years' service and a salary of £15,410 would receive £23,115 as a lump sum and £7,705 index-linked annual pension. Total cost to the university would be £39,989.

The vice-chancellors say that existing

early retirement terms for academics aged 50-55 years are unfair. Accordingly, they recommend an additional lump sum payment to compensate for up to five years' loss of pension rights. Hence a redundant academic aged 50 with 25 years of service and an annual salary of £15,410 would receive a lump payment of £21,566 in addition to an index-linked pension of £6,742 a year and a further lump sum of £20,226. The total cost to the university would be £72,150.

Academics under 50 years of age would be entitled to a lump sum severance payment in addition to the normal lump sum and pension payable after retirement age. The total extra cost to the university would be only the lump sum payment. This would mean that an academic made redundant at age 40 years with 15 years of service and an annual salary of £11,425 would receive £23,800 on redundancy, a £6,426 lump sum and an annual pension of £2,142 after normal retirement age. The extra cost to the university would be £23,800.

The vice-chancellors' committee hopes that the UGC will adopt its scheme when deciding how to compensate universities for redundancy payments. But precisely how much money the UGC can make available remains uncertain. The £20 million already earmarked will almost certainly be inadequate, but much will depend on when the bills come in.

The vice-chancellors' scheme, however, is being given short shrift by the Association of University Teachers (AUT), the academics' trade union, which refuses to discuss the possibility of compulsory redundancy. The AUT believes that universities should make economies only through early retirement, frozen posts and non-payroll savings. It is adding its voice to requests that the government should spread the cuts over five instead of three years.

The AUT's threat to start legal proceedings at the first hint of compulsory redundancy, even before names are

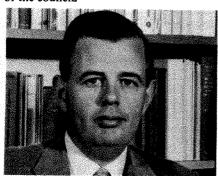
named, has already had an effect. Pressure from the local union branch at the University of Aston in Birmingham persuaded the university to postpone compulsory redundancies at least until the next academic year. Local pressure also persuaded the University of Keele to withdraw its notification of 300 impending redundancies to the Department of Employment. Other universities have also decided to delay job losses in an attempt to avoid being the first to be dragged through the courts. A handful are also apparently planning not to balance their books for several years. Their policy of living on borrowed money perhaps for three or four years is favoured by the AUT.

Judy Redfearn

# Science research council

# Paying a price

The capacity of the Science and Engineering Research Council to make research grants to British academics is likely to be impaired, in the year immediately ahead, because of the overspending by the council's Science Board, in the financial year that ended on 31 March. One of the ironies of this situation, described in the council's annual report for 1980–81, published last week, is that the chairman of the offending board, Professor John Kingman, has now succeeded Sir Geoffrey Allen as chairman of the council.



Kingman - poacher to gamekeeper?

According to the report, the council's allocation of funds to the science board will be reduced in the current year, while the board must repay to its master the outstanding amount of its over-spending.

The over-spending occurred, according to Professor Kingman, when the council decided to adopt a more bullish attitude to its spending to avoid falling into the trap of having money that it had not spent taken away by the government. In the event, it over-stepped the mark and received a sharp rap over the knuckles from the House of Commons Public Accounts Committee and a warning not to over-spend again.

Professor Kingman is confident that the council can do better this year. He expects spending to be within 0.15 per cent of the budget, the margin allowed by the Treasury, which he nevertheless complains is too narrow and would never be expected

in industry.

Although the science board over-spent by only a small fraction of its annual budget (£64.4 million in 1980-81), the effect on its activities has been marked. During 1980-81, the announcement of some research grants to universities had to be delayed for lack of funds. The repayments this year and next will also not help the board meet its future commitments, about one-third of which are to research and teaching in universities.

According to the annual report, support for university research, a third of which is channelled through the science board, had to be cut somewhat during 1980-81. Matters this year are complicated by the cut in university income which is increasing pressure on the council's research grants.

But the report, for which Sir Geoffrey Allen is responsible, says that spending on research grants should soon be restored at least to the level of recent years. Nevertheless, it is unlikely that the council will be able to support all grant applications given its highest merit grading of alpha.

The council, in 1980-82, spent £56.5 million on research grants out of its total budget of £207.8 million. A few more grants were awarded than in the previous year but the increase was due almost entirely to an unusually large number of requests for continuing support in some areas. A total of 1,983 research grants, valued at £74.5 million, were recommended in 1980-81 compared with 2,412 awarded in 1979-80.

Judy Redfearn

# New man in

Within a week of the resignation of the president and director-general of the French research agency, the Centre National de la Recherche Scientifique (CNRS) (Nature 5 November, p.3), a new director-general has been appointed: the long-time socialist party member M. Jean-Jacques Payan, a 46-year-old mathematician, and president of the University of Grenoble I.

The presidency of CNRS remains unfilled, although constitutionally the president has to help select the directorgeneral. However, the director-general bears the main management load, and so the filling of this post was considered the more urgent.

M. Payan's first task will be to reestablish confidence in the CNRS management, to represent CNRS in the national colloquium on science and technology (which takes place in January) and to consider the reorganization of CNRS — particularly taking into account the strongly expressed wish of the engineers, technicians and administrators of the many CNRS laboratories to play a more significant role in policy-making.

**Robert Walgate** 

# German energy policy

# The future unclear

Count Otto Lambsdorff, the West German economics minister, was balanced on a precarious political tight-rope last week, when he announced the governing coalition's energy policy for the next decade. Judging by his description of the "third continuation of the energy programme" (the second continuation was in 1977), he had little money, little energy and few plans. Or at least he had few plans he would admit to, for fear of rocking the coalition.

For example, to prepare the third continuation, Lambsdorff had asked several German economic institutes to make forecasts of energy needs and production. Usually a government will give guideline projections for the total economy in such circumstances; but Lambsdorff could not or would not provide them. And while the institutes clearly recommended a quadrupling of nuclear power generation by 1995 (from 3.7 per cent of primary energy to 17 per cent), Lambsdorff would only commit himself to the remark that "nuclear energy must provide an increasing percentage of baseload electricity'

Several factors lie behind this imprecision — strong local opposition within the Social Democratic Party (SDP), one of the coalition partners, to the expansion of nuclear power; the possible retirement of Chancellor Schmidt through ill-health before the 1984 general elections, complicated by the lack of an obvious successor; and the forthcoming fight in the local government elections next year, when SDP must present as united a front as it can.

Were it not for these political complications, the government would no doubt like to commit itself firmly to cheap nuclear power, which - behind the scenes - it sees as essential for maintaining the competitiveness of German industry. But if nuclear power is to be cheap, it must be plentiful, goes the calculation. A new German 1,300-MW pressurized water nuclear power station would cost nearly twice as much in Germany as in France, a German manufacturer claimed recently. According to the company, this is largely because the French companies were "tooled up" for long production runs, and environmental regulations and delays are less extreme in France. At best, nuclear power might contribute 25 per cent of German electricity by the mid-1980s, compared with France's 70 per cent by 1990, the company estimated.

Nevetheless, it is not good politics in SDP at present to be seen to be a strong supporter of nuclear power, in spite of the fact that there seems recently to have been a change of mood among the German public. Germany's strong dependence on oil and gas keeps domestic energy prices high, and the nuclear industry's claims that

nuclear power can be cheap is winning adherents.

Last week, however, Lambsdorff did commit himself to one change of policy. Like all Western ministers, he needs to save money during the world recession. Some of it will come from the coal industry—heavily subsidized in Germany to make its price competitive with the imported variety. These subsidies will be reduced, and cheaper coal imported. Germany should also shift away from burning "brown coal", and use it to provide chemical feedstocks. The missing energy would be provided by nuclear power.

The German government would not be deflected from its commitment to a DM 10,000 million (£2,400 million) deal with the Soviet Union to provide Western Europe with natural gas through an overland pipeline from Siberia, Lambsdorff said last week. The deal would provide West Germany with around 12,000 million cubic metres a year, 30 per cent of its natural gas requirements. A large fraction of this gas will be used to produce electricity. Natural gas power stations at present produce 50 per cent more electricity in Germany than do nuclear stations.

Robert Walgate

# **UK** Government Chemist

# New broom

The Laboratory of the Government Chemist, for long a respected but retiring establishment, may yet emerge from the shadows. For Dr Ronald Coleman, who will take over as Government Chemist when Dr Harold Egan retires at the end of December, intends that the laboratory should play a more central part in British science.

The laboratory, which employs about 400 people, provides a wide range of analytical services, mainly for the public sector. Thus it is concerned with the estimation of additives in foods and the characterization of oil-spill samples for correlation with suspected sources of pollution. Although supported by the Department of Industry, the laboratory is paid for specific services by government departments and local authorities. The laboratory has few dealings with the private sector and its research is largely that required to keep abreast of new analytical techniques.

Dr Coleman plans to change all this. He intends to broaden the remit of the laboratory, to make it "more open" and to

# Asian mycotoxins

In the story "Yellow rain: Waiting for data", Nature 22 October p.598, it was mistakenly stated that a sample of vegetation had been taken from Laos for analysis. In fact it was taken from Kampuchea.



Coleman - talking change

forge links with both industry and the universities.

Dr Coleman would like the laboratory to "have a finger in more pies". In its analytical work he would like the laboratory to branch out into work on surface chemistry and on-line instrumentation. Mr Coleman himself has just been given responsibility for biotechnology in the Biotechnology Directorate set up last month, but the laboratory's role in this new venture remains to be determined.

Dr Coleman is also ambitiously planning coordinated programmes with industry, hoping that the laboratory could contribute technical expertise and industry the necessary funds. He has already talked to five interested industrial companies.

Dr Coleman points to his varied experience. At present he is deputy director of the National Physical Laboratory, but his career began with five years in the glass industry. His twenty-year spell with the United Kingdom Atomic Energy Authority involved work with nuclear energy, forensic science, geochemistry and biotechnology.

Isobel Collins

# Polish science

# Academy for change

Warsau

Self-governance, the move towards "social control" of all sectors of public life, will undoubtedly bring considerable changes in the structure of Polish research planning. In particular, there have been vocal demands, coming especially from the scientists' lobby within the free trade union Solidarity, for parity of status between the universities and other higher educational institutions, the Academy of Sciences and its subsidiary institutes, and the institutes belonging to the production ministries (including the ministries of agriculture, fisheries and mining).

Funding tends to be less generous in the academy institutes than in those belonging to the ministries, many of which, it has been suggested, were founded more for reasons of prestige than for any real

research need. Although with Poland's newly-admitted unemployment problems—particularly among young graduates—closures are unlikely, a thorough-going reconsideration of the pattern of research is expected. Any moves from the top to start making changes would, however, run the risk of conflicting with the demand—expressed by the recent Solidarity congress—that scientists should have more say in choosing their own pesearch topics.

Another Solidarity recommendation calls for the elimination of the barriers between the academy and the rest of the academic community. During the past few years the various academy institutes have become havens for young academics prevented from taking teaching posts for political reasons, and one of the changes suggested by Solidarity would be to allow scientists working at academy institutes to lecture to university students.

At the same time, there is considerable unrest both inside and outside Solidarity about the present anomalous status of the academy as a quasi-ministry, with its academic secretary responsible not to the academy but directly to the prime minister. A spirited debate, on this and other points, is expected when the general assembly of the academy meets in December, chiefly to debate the proposed new legislation on the academy.

A new bill on higher education is already on its way to becoming law, and (following the protests and strike-threats of last September) it now has the basic consent of the academic community. Vera Rich

# Californian Medflies

# **Brief armistice**

Sacramento, California

Aerial spraying against the infestation of the Mediterranean fruit fly, now much curtailed for the duration of what Californians laughably call the winter, is likely to be resumed in earnest in the spring. But Mr Richard Rominger, director of the state Department of Food and Agriculture, told his Advisory Committee on Thursday last week that enough insect traps have been installed in the northern counties for next year's campaign to be effective.

Meanwhile, fruit farmers in Florida and Texas as well as California are more concerned with an unexpected consequence of the Californian campaign against the Medfly. The practice of fumigating fruit from affected areas of California with ethylene dibromide has raised environmental objections in Japan, one of the chief markets for United States fruit exports, and has also drawn attention to widespread use of ethylene dibromide for fumigating fruit from Florida and Texas (where other pests, such as the Mexican fruit fly, are common). Mr Rominger was hoping, last week, that the Japanese authorities would now accept as safe the Californian requirement that fumigated fruit should contain no more than 130 parts per billion of ethylene dibromide, but he had yet to persuade longshoremen in the United States that fumigated fruit could be handled safely.

Nobody knows the economic cost of the "abrupt interruption of the channels of trade" caused by the infestation and the consequent fumigation, but Mr Rominger thinks it exceeds by an order of magnitude the direct cost of aerial spraying to California, put at \$50 million for this year.

The reasons why California was caught napping by this year's events are now becoming clear. Operations against the Medfly are directed from the state's laboratory at Los Gatos, between San Jose and Santa Cruz to the south of San Francisco Bay. Medflies were first recognized in Southern California in June 1980, but that infestation was successfully dealt with by the release of sterile male flies, as had been the 1975 infestation near Los Angeles.

The importance of the nearly simultaneous outbreak in Santa Clara county (just north of Los Gatos) was appreciated only much later, partly because insect traps were more thinly spread but also because the complete life-cycle of the insect (22 days in tropical conditions) seems to vary enormously from one microclimate to another and may be as long as 77 days in the northern counties.

The density of traps in the northern counties at risk has now been increased to 50 per square mile, and adult Medflies are still sporadically being caught in them. Members of the laboratory's staff recall with anguish the early months of this year, when they were "desperate" for supplies of sterile males to release into the field. More than 10,000 million were used before aerial spraying was begun in mid-July.

The belief that supplies of supposedly sterile larvae from Peru were in reality fertile seems not to be supported by the evidence the laboratory has collected. The staff says that the Peruvian supplies of sterile flies were, however, poor in quality — some consignments yielded only 10 per cent of viable adult flies. The fertile females of Peruvian origin recognized in the field in June this year (on the basis of a fluorescent dye) may well have derived from larvae contaminating a Peruvian consignment after the bulk of it had been irradiated.

The present hiatus in the campaign against the Medfly has not stilled political recriminations, directed chiefly at Governor Edmund G. (Jerry) Brown. Mr Brown's stand against the aerial spraying of a carbohydrate bait laden with malathion, in the face of technical advice to the contrary, is widely resented by his opponents in the state legislature as well as by farmers' organizations and many agriculturalists.

Thus Mr Richard Niellson, a member of the State Senate, is concerned that the incident will have permanently damaged California's reputation as a reliable supplier. He, among other members of the legislature, is planning to use the budgetary process now beginning to see that proper provision is made for the war against the Medfly in 1982 and succeeding years. But this year's experience, when the Red Cross centres set up to deal with casualties among the urban population of California were hardly used, has, Mr Niellson says, seen a decline of the "Rachel Carson syndrome" among the Californian electorate.

Mr Rominger, who with some of his technical advisers had threatened to resign if aerial spraying was not begun, hopes that the 17-member "pest prevention task force" due to hold its second meeting this Monday will insulate pest protection from politics. The committee is intended to present the governor and the state legislature with a general strategy for protection against all insect pests by the beginning of 1982. Meanwhile, the state will have to decide what to do about the 700 claims for civil damage which have been lodged, while inspection stations at the Californian border are likely to reappear.

For most Californians, the events of the past months are likely to prove cathartic. Next season, farmers (who are not prevented from ground spraying with malathion and more powerful insecticides) will take more active steps to protect their crops. The state as a whole seems to have recognized the economic importance of its agricultural industry, worth \$14,000 million a year. And people in urban areas have been left with a lasting impression of helicopter visitations at night (when the air is still), with their searchlights illuminating suburban gardens. "This is what it must have been like in Vietnam" said one whose white Volkswagen is still spattered with grey malathion bait.

# **Nuclear Structure Facility**

# Nylon slip shows

Water in the nylon insulating links of the charging system of the Nuclear Structure Facility, a 30 million volt tandem Van de Graaff accelerator for nuclear physicists under construction in the United Kindom at Daresbury Laboratory in Cheshire, is now blamed for delays which will put the commissioning of the machine back at least to spring 1982.

A new material, Torlon, will have to be used in place of the monocast nylon in the pioneering machine, which is the highest voltage Van de Graaff yet designed. The nylon, Daresbury physicists found to their cost, contained a small proportion of water. This did not affect the insulating properties of the nylon at room temperature, but the conductivity rose a hundred-fold between 20°C and 35°C, the physicists discovered. So when the "laddertron" — a charging belt like a tank track made of alternating links of nylon and aluminium to carry charge up to the high-voltage

terminal — was first tested at full scale, and friction raised its temperature to 35°C, it failed.

Torlon, an American-made material, was not available when the accelerator was first designed, but in a test section of laddertron it has proved its superiority to the monocast nylon. The whole laddertron will now be rebuilt with Torlon.

However, the problems of the accelerator are not over yet. Unfortunately monocast nylon was used for other parts of the accelerator. Now the laddertron problems appear to have been solved, these too may have to be replaced.

Moreover, early next year the accelerator tube — the evacuated metal and ceramic tube down which the beam of nuclei must pass — will be installed and tested. And unless the Daresbury team are lucky for once, this too may cause delays. At Oak Ridge National Laboratory in the United States, problems with a similar tube set a 25 million volt machine back by a year — and even now the laboratory may have to be content with 20 million volts rather than the design figure.

Nevertheless the delays at Daresbury have had one bonus. All the experiments will be ready in time for the first beam, despite a low rate of funding which implied that only the bare minimum of experiments would have been possible earlier.

Robert Walgate

# Caltech

# Kellogg birthday

Pasadena

The Kellogg Radiation Laboratory at the California Institute of Technology combined its celebrations of its fiftieth anniversary last week with the dedication of a new custom built Megavolt accelerator and a seventieth birthday party for Professor William Fowler, the third director of the laboratory. Fowler, always ebullient, stole the show, but the laboratory was at pains to emphasize that it has been clever enough to equip itself with a new accelerator, albeit in the MeV range, when high-energy physics laboratories are wondering how long their GeV machines will be able to function.

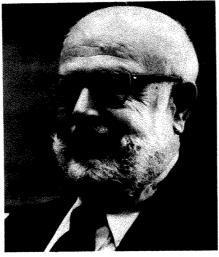
The Kellogg Laboratory is the West Coast's chief monument to R. A. Millikan, the president of the California Institute of Technology when the laboratory was first formed. Millikan's original prospectus included the use of high-energy radiation in the treatment of cancer. During the Second World War, the laboratory played an important part in the determination of nuclear cross-sections needed for the Manhattan Project. Since then, it has been principally concerned with the application of nuclear physics to other fields, astrophysics in particular.

The new accelerator, represented last week by its external yellow tank and christened as if it were a sea-going object, has been designed for high ion current and voltage stability. It will be used, among other things, for the laboratory measurement of nuclear cross-sections relevant to the processes of nucleogenesis which are at present uncertain.

But Dr Barbara H. Cooper also outlined a scheme for using the accelerator as a charge spectrometer for checking the claim by William Firbank and his colleagues at Stanford University that electric charges exist which are a fraction of the electronic charge, and which might represent free quarks. Preliminary estimates suggest that by using an argon beam for sputtering free atoms from solid surfaces, it should be possible to evaporate one of the small niobium spheres used in the Firbank experiments in about an hour, and that the tandem accelerator should be capable of detecting one fractionally charged particle in as many as 10<sup>18</sup> niobium atoms.

While astrophysical problems will remain a central part of the Kellogg Laboratory's programme, diversification is also in the air. This is the spirit in which the laboratory is involved with studies of the release of radon-222 as possible predictors of major earthquakes, just now a matter of public interest in California.

According to Professor Thomas A. Tombrello, two of the network of radon monitors maintained by the laboratory near the San Andreas Fault had revealed a substantial increase of radon evolution since the early weeks of August. The



W.A. Fowler at seventy.

increased rate of evolution (which continues) has been closely correlated in time even though the two instruments are separated by 100 kilometres, and is said to resemble that which preceded the 1979 earthquake of magnitude 6.7. That event was marked by a transition from the normal compressive stress across the surface rocks of the San Andreas Fault to an extension — thought to stimulate radon release — but also by a cessation of radon release in the days immediately before the earthquake.

Professor Tombrello prudently avoided prediction last week, saying only that "sometimes, perhaps in 25 or 50 years, one

of these events is going to be a precursor of a major earthquake". He complained, however, that financial support for earthquake studies of this kind in California was less than that in China and Japan. "Since 10 million people would be affected by a major earthquake, it would be worth putting a little money into it."

# Albanian development

# Hoxha looks ahead

Albania, too, has an urgent need of science and technology, according to party leader Enver Hoxha's speech to the congress of the Albanian Workers' Party last week. But although the "deepening of the technical scientific revolution" has encouraged teaching and research, practice lags behind precept. Mr Hoxha said that the most obvious need is for a mechanism for the gradual transfer of innovation on a "wider front" aimed at the "radical" transformation of technology and production.

Mr Hoxha's criticism came in a speech praising the achievements of Albania's system of education, scientific education in particular. He drew particular attention to Albanian achievements in hydroelectric and railway engineering, the sinking of deep wells, geological prospecting, stockbreeding and the machine tool industry. He emphasized that these had been accomplished by the Albanian people "relying completely on their own forces" justification of the country's long standing isolation policy. Although the Academy of Sciences is still the major centre for scientific research in Albania, Mr Hoxha said that the Committee for Science and Technology set up this year was an important instrument for the "better direction and organization" of science.

The targets for the Albanian 1981–85 five-year plan are more ambitious than in any previous quinquennium. Industry will receive some 46 per cent of the total investment budget, with special emphasis on mining and energy resources. Oil extraction is expected to rise by some 60 per cent, coal by 48 per cent and that of proved mineral resources (chromium, copper and iron nickel) from 30 to 200 per cent. The chemical industry will be considerably expanded, with an expected rise in production of some 65 per cent by 1985.

Mr Hoxha was at pains to say that these targets are "scientifically based and fully achievable". Furthermore, to provide the necessary personnel, special attention will be given to higher education. Student admissions during the next five years will rise by 45 per cent, new courses will be introduced and there will be a considerable expansion of postgraduate courses. Publication of "political, scientific, technical and artistic" books will rise by one million copies over the previous quinquennium and educational cinema, radio and television will be expanded. Vera Rich

# Hoyle on evolution

The serious part of the Kellogg symposium provided Sir Fred Hoyle with an opportunity for a moderate (and self-critical) statement of his case for disbelieving conventional views about the evolution of the Universe, the "big bang" among them. Hoyle has been associated with the Kellogg laboratory since his collaboration in the mid-1950s with W.A. Fowler and the two Burbidges (Margaret and Geoffrey), now known as the gang of four, on the problem of nucleogenesis.

Hoyle said last week that, although content in the mid-1960s to give the supposed connection between the microwave background radiation and the big bang a "good run for its money", he had now lost patience with this approach. Two of his reasons involve the origin of life - the calculated time since the origin of the Universe of 10,000 million years or so is not enough to account for the evolution of living forms, while adiabatic expansion of the Universe would have been inimical to the evolution of highly ordered forms. But Hoyle also said that new evidence in support of the big-bang hypothesis was emerging only slowly. Yet "when people are on the right track, new facts emerge quickly". Hoyle said he would change his view if it turned out that neutrinos have a mass of between 20 and 30 electron volts.

The essence of his argument last week was that the information content of the higher forms of life is represented by the number 1040,000 - representing the specificity with which some 2,000 genes, each of which might be chosen from 1020 nucleotide sequences of the appropriate length, might be defined. Evolutionary processes would, Hoyle said, require several Hubble times to yield such a result. The chance that higher life forms might have emerged in this way is comparable with the chance that "a tornado sweeping through a junk-yard might assemble a Boeing 747 from the materials therein".

Hoyle acknowledged that steadystate theories of cosmologies, of which he was one of the chief exponents in the 1950s, are not now tenable because of the evidence for evolutionary galactic and stellar processes. But the big-bang view is similarly not tenable because of the way in which it implies the degradation of information. Of adherents of biological evolution, Hoyle said he was at a loss to understand "biologists' widespread compulsion to deny what seems to me to be obvious".

# CORRESPONDE CE

# Good as gold

SIR — In the current debate on a gold standard the stumbling block is the fundamentally wrong premise in the quest to fix a price. It is not possible to fix a price for gold in terms of any existing currency. Far better to consider not a price but a value. Gold has a value as a medium of exchange, in other words as a currency. Consider the implications of using gold as money.

Assume all gold reserves minted into coins of standard weight and fineness. At a given date equate the number of coins so available with the figures used internationally in representing the price of goods and services in circulation. One coin will be found to equate to many hundreds of dollars.

Now start using only gold coins to pay for goods and services. Note the dramatic fall in the numbers on the price tickets (the first skirmish in the war on inflation). Continue to mint all newly-won gold into the standard coins. Charge the weight of gold going into industry or jewellery at the value of the equivalent number of coins. Paychecks would of course come down accordingly. Cheques and all paper transactions could be continued, but only against deposits in the equivalent number of coins. Gold money thus on deposit would pay interest whereas gold now in the bank does not.

The result would be a stable currency and stability of values. The gold-producing countries wold not get any richer (you can't eat gold) whereas countries with exports of commodities or manufactures, instead of suffering from "lack of hard currencies" would find their products are as good as gold.

This desirable state of affairs would continue as long as the supply of coins was adequate to satisfy the needs of international commerce. However, the rate of increase of the net worth of the world is historically greater than the rate of increase of gold production and is likely to remain so. In this situation the remedy would be to have a periodic revaluation of the value of the coin. Clearly this value is going to tend upwards and, as a concomitant, the numbers on the price tickets will go down — the very reverse of inflation. Gold thus produces capital gains.

The return to gold usage (rather than a gold standard) would provide a solution to currency and exchange problems and, since the money supply would be finite, a cure for inflation and a clear directive for monetary policy.

JOHN H. HARRIS

New York, USA

# Harsh words

Sir — Your comments on Dr Rupert Sheldrake's book A New Science of Life (Nature 24 September, p.245) are very harsh. One can hardly pretend that our current understanding of development and its genetic control is adequate. We should not even believe that it is oriented in the right direction. The concept of morphogenetic fields is neither unscientific nor without support from some distinguished embryologists. If the same concept is extended to all aggregations of matter, animate or otherwise, it may be considered as a bold, hazardous, foolhardy or

unwarranted generalization, depending on one's attitude towards new thought. Just a hundred years ago if somebody suggested that the sequence of nucleotides in DNA constitutes the information required to make a fly, a frog or a man from a small bit of cytoplasm it would have been dismissed as preposterous. If there is no obvious way of testing experimentally Dr Sheldrake's hypothesis of formative causation, it does not preclude its possibility in the future.

Many embryological theories depend on recourse to morphogenetic fields in spite of the recent progress in genetics and molecular biology. No real progress can be made in understanding the importance of these fields without identifying the nature of the variables along the gradients. Perhaps the only measurable variable to which the origin of a gradient can be traced is the concentration of diffusing substances. Positional information which adds a new dimension to the concentration of diffusing substances is also now accepted as a fundamental mechanism of morphogenesis. However, even these powerful theoretical tools have not successfully accounted for any single embryological event. In fact it would be preposterous to assume that development of complex organisms can be explained solely on the basis of the current theoretical platform on which molecular biology stands. Recourse to new conceptual framework and unconventional mechanisms is imperative to understanding complex biological processes such as morphogenesis.

K. Vasudeva Rao

University of Zoology, University of Delhi, Delhi, India.

# Page charges

Sir - Frank Close's review of high-energy physics journals in Nature 1 October (p.369) reveals a misconception that may be widespread enough to warrant correction in public. I refer to his statement that "in times of financial stringency European authors are often unable to publish there [in Physical Review] as a result of page charges". The fact is that the page charge system, not only in Physical Review (including Physical Review Letters) but in all journals published by the American Institute of Physics, is a voluntary one. Payment of page charges is requested. but acceptance of a contribution is not conditioned by whether page charges are paid. The only effect of nonpayment is a delay in publication; and even that is not imposed on papers in Physical Review Letters, or on the newly instituted rapid communications section in Physical Review.

I hope I will not be misconstrued as urging authors to submit papers to our journals and not honour the page charges when they are in a position to pay them. I do not even mean to solicit papers for the journals. Those costs that are not met by page charges have to be made up from higher subscription costs for persons and institutions that are not members of the various societies; and we are as concerned as anyone else over the effects of ever higher costs to libraries. I do feel, however, that it is important that the true state of affairs be completely understood.

George L. Trigg

GEORGE L. TRIGG (Editor)

Physical Review Letters, New York, USA

# Belgium's power

SIR — Your recent article on nuclear safety in Belgium (*Nature* 24 September, p.249) prompts me to explain some points about Belgium's approach to nuclear power.

First, the nuclear power station licensing procedure in Belgium involves two stages: (1) on approval of all planning and logistical aspects and the safety guidelines, permission is granted for construction to start and (2) after subsequent safety analysis and approval, permission is granted to put the power station into operation.

During plant construction there is continued liaison between the public authorities, technical experts, suppliers and the station owner, to ensure that all safety aspects are in line with the Belgian regulations. This process involves the exchange of about 2,000 questions and answers, and has the advantage that the most modern safety innovations are incorporated into Belgian nuclear power stations at an early stage. This could clearly not happen if the design had to be frozen at the time of the construction permit.

Given the long construction period required for such plants, this procedure eliminates the problem of premature technological obsolescence. It is also interesting to note that Belgium is the only country to submit nuclear power station designs for approval to a committee of international (EEC) experts.

Second, the question of the number of specialists officially appointed to deal with nuclear safety was somewhat misrepresented in your article. Belgian law confers supervisory authority for the nuclear sector to a state-approved inspection organization, Vinçotte. Other official government bodies, however, exercise ultimate control in this field. So in fact there are many more than the 17 Vinçotte inspectors involved in supervising the safety of the Belgian nuclear power stations.

On the other hand, although it is true that the International Atomic Energy Agency has cited the figure of 50 experts as a standard force to deal with nuclear safety in a country like Belgium, the responsibilities of such a force would include operational control, regulatory missions, evacuation plans and so on, in addition to overseeing general safety precautions. In Belgium these aspects are not neglected, but are properly dealt with by the governmental bodies and technical experts mentioned above, (including the "Commission for questions concerning radiation").

Finally, with respect to the threatened strike by the employees of Vincotte, their qualms did not stem from questions of power plant operations, but rather from the uncertainty of their career prospects.

So to summarize, (1) the procedure followed by the utilities before putting a nuclear power plant in operation strictly follows Belgian law—the strictest in the world, according to IAEA. And (2) the force of experts and inspectors in charge of safety supervision is perfectly adequate, both in number and competence, and is in a position to enforce the nuclear safety regulations, as it has indeed done (nuclear power stations have been operating in Belgium for 20 years).

R. VAN DEN DAMME

Societé Intercommunale Belge de Gaz et d'Electricite, Brussels, Belgium

# NEWS AND VIEWS

# High energy physics grows tenfold

from Robert Walgate

THE first ever controlled collisions of fundamental particles at centre of mass energies of over 540 GeV — ten times higher than previously achieved, and an energy at which spectacular physics is expected — are beginning to be observed at the new proton antiproton collider at the European centre for nuclear research (CERN), even before the machine is out of the hands of its constructors.

"We take data while the engineers take their coffee breaks", Carlo Rubbia, leader of one of the two experiments now in place, said last week. Rubbia - a well-loved Italian-American maverick of high-energy physics - in fact proposed the whole idea of the collider, in which bunches of protons at 270 GeV collide with oppositely moving bunches of antiprotons. The proposal came in, in a report at the 1976 Aachen neutrino conference titled "Looking for the intermediate vector boson with existing accelerators". He has not been slow to reap the rewards now the machine is in place. So far his experiment (UA1) has recorded some 20,000 events, but no intermediate vector boson.

On the other side of the collider (which is in fact the super proton synchrotron modified to take colliding beams) is experiment UA5, headed by Dr John Rushbrooke of the University of Cambridge with professors Klaus Bockmann of Bonn, Jacques Lemonne of Brussels and Gösta Eksborg of Stockholm and their groups. UA5 has taken 1,500 pictures (one of which appears below) with another 10,000 expected in the next month.

The events of UA1 and UA5 together are only the first scratching of the surface of physics at these exalted energies, but already there are some indications of what may or may not be expected.

The physics needs to be seen in relation to two quite separate classes of prediction. On the one hand, there are some peculiar data from cosmic ray experiments — a handful of "Centauro" events and others — which indicate that there may be a substantial change in physics at energies close to, or perhaps somewhat above, the energies of the new collider. On the other, there are the predictions of the Salam-Weinberg unified gauge theory of the weak and electromagnetic interactions, which has been well-tested at lower energies in electron and neutrino scattering experiments.

This theory is the backbone of most current attempts at a "grand unification" of all the forces, and it predicts the existence of three intermediate vector bosons (Z°, W+, and W-) at masses of around 80 GeV. These should be created at collider energies, and should be seen to decay as predicted by the theory. The theory also predicts new scalar particles called Higgs' particles, but the masses of generally speaking, these are. undetermined and the particles may or may not turn up in collider experiments.

So far there are no data from CERN on the intermediate vector bosons, but that is not unexpected. Estimates suggest that the IVBs will only be produced in small numbers, and too few events have been collected so far to expect one among them. Finding the bosons also depends on increasing the luminosity of the collider (effectively the number of collision per second per unit interaction cross section) by orders of magnitude beyond those presently being reached at CERN. Design luminosity was  $10^{30} \, \text{s}^{-1} \, \text{cm}^2$ ; the present achievement is  $2 \times 10^{25}$ . A luminosity of  $10^{29}$  would give Rubbia about 10 Ws and

1-2 Z° a day, he estimates, allowing for his detector efficiency.

CERN machine physicists have however not yet attempted to "tune" the collider to give its maximum luminosity. Two effectively independent factors of 50 could be obtained relatively easily, it is believed, which would bring the machine within a factor of 2 of a luminosity of 10<sup>29</sup>. The remaining factor of 2 might be made up within a year, machine physicists feel, with 10<sup>30</sup> remaining so far a very distant goal.

More easy to check are the cosmic ray results. These indicate that some extraordinary and inexplicable interactions may set in at centre of mass energies of the order of 1,000 GeV (This is around twice the collider energy, but the cosmic ray experiments have a detector threshold at about that level. Rushbrooke believes, so the physical threshold may be lower.) These Centauro events - of which only five or so have been observed, cosmic ray intensities being so low at this energy - show the production of very large numbers of charged particles (track multiplicity) and a mysterious near absence of neutral pions. Also the average transverse momentum of these and other high energy cosmic ray events rises with multiplicity, something quite unknown in lower energy data.

However, so far neither UA1 or UA5 have found any clear indication of these phenomena at the collider — though exact results await more precise data analysis. Confirmation of the cosmic ray results may have to wait for higher statistics — or for a collider of even higher energy, such as the 2,000 GeV centre of the mass energy collider being planned at Fermilab near Chicago for action in 1984 or 1985 (Ronald Reagan willing).

Robert Walgate is Chief European Correspondent of Nature.

One of the first 540 GeV p-p events to be recorded in the Cambridge-Bonn-Brussels-Stockholm streamer chamber (experiment UA5), showing clear forward and backward cones of tracks. The streamer chamber is the only detector on the collider to record particle tracks clearly close to the interaction point. It will provide a first survey of the physics of interactions at these new energies.



Latters, C.M.G., Fujimoto, Y., & Hasegawa, S. Physics Reports 65, 3 (1980).

# How cells live together

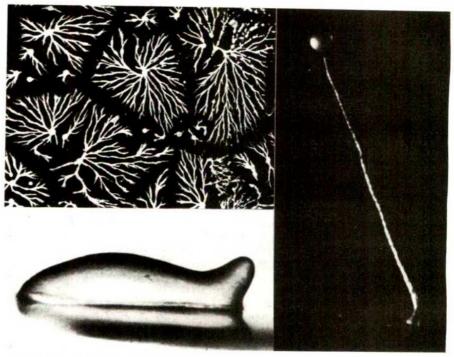
from Robert Kay

THE cells of a higher organism normally behave in a completely social way; it is the occasional deviant - such as a cancer cell - that attracts our attention. But how is cooperation between cells in a multicellular organism established? The problem is clearly seen in the cellular slime moulds, such as Dictyostelium discoideum. They feed as free-living amoebae, but when starved the cells aggregate by chemotaxis (to cyclic AMP) forming a small multicellular organism. This 'slug' though without the benefit of nerves or sense organs, can orientate and move towards light or heat. Eventually it differentiates into a fruit, with a stalk supporting a mass of spores. It was apparent from a recent meeting\* that to establish multicellularity cell-to-cell adhesion (and production of a sheath around the organism) and communication systems to control both movement and differentiation are necessary.

Cohesion is thought to be brought about by any of four systems in Dictyostelium: through contact sites A and B (cs A,B), by the discoidin lectins and by a 95K membrane protein from slug cells1. Appropriate antibodies and treatments can block cohesion via each system and now the pressing problem is to establish their respective roles in vivo. Progress is being made using cohesion-defective mutants. In a ts mutant described by Sussman (University of Pittsburg) cohesion during aggregation is normal at the restrictive temperature but is lost abruptly at the time slugs would form in the wild type. This indicates that the aggregative cohesion systems are replaced by a new mechanism in the slug. As the 95K protein does not appear at the restrictive temperature, it probably is part of the new mechanism; analysis of wild-type membrane extracts that restore cellular cohesion to mutant cells should test this supposition. Mutants affecting cs B, on the other hand, develop quite normally showing that cs B has only a minor role in developmental cohesion (Vogel, Max-Planck-Institut, Munich); its major function is probably in binding bacteria preparatory to phagocytosis and in cell-to-substratum adhesion. Similarly, in a related slime mould species cohesion is unaffected by mutation of its lectin (Francis, University of Delaware), so that by elimination, cs A is probably responsible for aggregative cohesion.

The signal coordinating forward movement of cells in a slug may or may not

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After a few hours of starvation *Dictyostelium* amoebae initiate and relay cyclic AMP signals which guide them into streams leading to collecting points, where the cells mound up (top left, in dark field). These mounds transform into migrating slugs, each up to about 2mm long (lower left). The slug has an anterior organising 'tip' and eventually it differentiates into a fruit consisting of a mass of refractile spores supported by a cellular stalk (right).

be cyclic AMP, but the steering of the slug towards light involves something new. In a model proposed by K. Williams (Max-Plack-Institut, Munich) differential illumination of the slug tip generates a transverse gradient of an extracellular signal molecule, slug turning factor (STF), in the slug, to which cells respond by a change in direction, most likely by chemotaxis. The properties of STF are consistent with this model: it is made in response to light, high environmental levels disprientate phototaxing slugs and it is itself a slug chemotactic agent. Interestingly, STF, the stalk cell differentiation inducing factor DIF (Kay, ICRF, London) and an inducer of fruiting2 are all low molecular weight, hexanesoluble factors; whether these similarities point to identity or close relationship is as yet unknown.

Cyclic AMP signalling controls both cell movement and gene expression (for discoidin, at the level of transcription; J. Williams, ICRF, London) and it is becoming apparent that the coupling machinery used is similar to that of some mammalian hormones. Thus, Dictyostelium adenyl cyclase has a GTP binding and cholera-toxin-sensitive component analogous to the mammalian G protein (Leichtling and Rickenberg, National Jewish Hospital, Denver), whilst the major and most specific cytoplasmic cyclic AMP

binding protein (of about molecular weight 40,000; Veron, Pasteur Institute; Cooper, University of Michigan; van Driel, University of Amsterdam) inhibits the catalytic subunit of mammalian cyclic AMP-dependent protein kinase (Rickenberg) indicating that it too is part of a cyclic AMP-dependent kinase, as reported some years ago<sup>3</sup>. Similarly the Ca<sup>2+</sup>-mediated effects of cyclic AMP signalling on myosin phosphorylation involves calmodulin (Moruta, Max-Planck-Institut, Munich).

It has long been suspected that coordinately regulated genes (such as those regulated by cyclic AMP) might be distinguished by a common control element (see, for example, ref. 4). Just such an element may now have been found (Lodish, MIT). It is a sequence 300-500 bases long, interspersed in the genome and transcribed into 50 or more messenger RNA species by the fifth hour of development, but absent from vegetative RNA. Different sequences mark other groups of mRNAs present later in development and in growing cells5. By a fortunate coincidence a powerful means for finding the function of these sequences is now at hand. Firtel (La Jolla) described the first efficient transforming plasmid in Dictyostelium which has a selectable marker with a suitable Dictyostelium promoter and origin of DNA replication,

<sup>\*</sup>The conference on Gene Expression and Membrane Changes in Cellular Slime Molds was organized by Gunther Gerisch and held at Tutzing, September 2-6, 1981 with support from the European Molecular Biology Organization and the Max-Planck-Gesellschaft

and can give transformation frequencies as high as 1 in 104 cells. It should now be possible to see what happens to a foreign gene introduced into the cell attached to one of the above putative control sequences. In principle it should also be simple to isolate genes essential for development by complementing developmental mutants with wild-type DNA inserted into the plasmid.

The Dictyostellum slug is a sort of freeliving embryonic field and there is great interest in how a regulative pattern of prestalk and pre-spore cells is established in it. The problem divides into two: what induces an amoeba to differentiate into a pre-stalk or a pre-spore cell and then what establishes the spatial pattern of cell types? From work with isolated cells it appears that cyclic AMP is required for both stalk and spore differentiation but that DIF can, by favouring stalk formation, control the

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Hermen, R.J. & Dievickoon, E. H. Schwicz 1803, 349 (1909) Kmmel, A.R. & Pittel, R.A. Cell 16, 787 (1979) Neture 291, 532 (1981) Le Rosth, D., Shilosch, J., Roth, J. & Lemsak, M.A. Proc. metr. Acad. Sci. U.S.A. 77, 6184 (1980)

choice of pathway (Kay). Several groups (Takeuchi, University of Kyoto; David, Munich University; Durston, University of Utrecht; MacWilliams, University of Massachusetts) strongly felt that sorting out of pre-stalk and pre-spore cells is an important way in which patterning is established (see ref. 6). However, we are a long way from knowing if this is the major or only way to generate pattern, especially as during regulation of pre-stalk isolates, and in D. mucoroides slug migration (where a stalk is continuously laid down), cells seem to differentiate according to their position (Gregg, University of Florida).

It is evident that even the lowly multicellularity of Dictyostelium is complicated enough for our present comfort. It would be nice to know that the tricks learnt by aggregating slime mould amoebae are still used (along with new ones) by higher and less tractable organisms. Of course, until both are understood this remains debatable, but recent evidence that many mammalian hormones7 and their response mechanisms are also present in simple eukaryotes suggests that tricks once learnt are not forgotten.

Unfortunately, in an amorphous specimen orientation is random and so unspecified, and even in a crystalline specimen the outgoing signal cannot be directed with any precision. However, the near-edge data involve rather slow and easily scattered electrons which will scatter between several atoms before eventually returning to the centre. Having travelled more widely than single-scattered electrons their phase contains information about the threedimensional disposition of the neighbours, but is much more difficult to interpret.

The limitation of information to radial structure, that is, to the radial distribution function, is a general property of weak scattering probes such as X-rays, neutrons or electron microscopes. It is particularly restrictive in systems where a knowledge of bond angles as well as bond lengths is important to an understanding of the structure.

Having proved the technique for well characterized materials the Daresbury scientists can be expected to tackle more exotic systems such as multi-component glasses or liquids, or biological molecules for which structural information is not available in advance.

The X-ray absorption experiments which have been first past the post in the race to publish are in fact only a part of the activities planned by university and industrial groups using Daresbury. In the first instance two beams of radiation are available. One provides for X-ray topography, and for X-ray interferometry, as well as for the absorption experiments referred to above. The other, providing radiation at longer wavelengths in the vacuum ultraviolet and soft X-ray range has two main experiments. The first VUV station to be operational measures the photoemission of electrons from surfaces and has the capability of resolving the direction as well as the energy of the escaping electrons. The second experiment measures the absorption cross sections of atoms adsorbed on surfaces and provides information in the surface context very similar to the structural information available for X-ray absorption experiments performed on both samples. Both these experiments will further our understanding of surface structure - important in catalysts, semiconductor technology and other areas of surface science.

In the near future more beam lines will become available, one providing for experiments in the area of atomic and molecular physics. Another will make available radiation in the infrared region for experiments on large organic molecules thus extending even further the range of wavelengths and types of application of the facility.

D.J. Thompson's team at Daresbury is currently completing the final stages of fine tuning of the SRS which now operates to specification with a regular schedule of users. Soon a major enhancement of the source will be made with the installation of

# Tripping the light fantastic

from John B. Pendry

THIS issue of Nature contains the first data taken on the new Synchrotron Radiation Source (SRS) housed at the Daresbury Laboratory. The source is a 2 GeV electron storage ring, with a design current of 370 mA, dedicated to the production of synchrotron radiation. Scientists at British universities and laboratories now have access to their own central facility for synchrotron radiation which will enable them to compete on equal terms with scientists in other countries.

The paper, by Greaves, Durham, Diakin and Quinn, (see p. 139) is an extension of the now familiar technique of using X-ray absorption fine structure to determine local crystallography [Sayer et al. Phys. Rev. Lett. 27, 1204, 1971]. The principle is that X-rays eject an electron from the inner core of an atom. The X-rays can be tuned to a particular type of atom because the inner shell ionization energies are strong functions of atomic number. When the ejected electron encounters the atomic neighbours of the excited atom it can be scattered and some of these secondary waves get back to the central atom. There, because the electron is described by a wave function, an interference process takes

place that may suppress or enhance the absorption cross section according to the phase of the scattered wave. Increasing the X-ray energy changes the electron wavelength and modulates the phase in a way that depends on the distance of the scatterers from the central atom. In this way X-ray physicists have discovered how to make a microscopic radar station. So far we are on well-trodden ground. What is new about the Daresbury paper? The experiments reported are X-ray absorption measurements on copper and manganese and the message is not so much an unravelling of the spectra of these materials as a jeu d'esprit of experimental and theoretical excellence demostrating that these spectra can be easily and accurately measured and, what is more, interpreted in a region of the spectrum, the near-edge region, which has previously been regarded as too complex to provide useful information.

The ability to interpret near-edge data goes some way towards removing a restrictive aspect of the X-ray absorption technique. For a radar station to give complete information about surrounding objects the precise orientation of the aerial must be known when the signal is sent out. Otherwise what emerges is merely a radial map with no directional information.

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a powerful superconducting magent (called a "wiggler" for technical reasons) which will accelerate the electrons in the source so strongly that the spectrum of radiation, currently cutting off at a wavelength around 0.3 Å, will extend down to 0.1 Å.

Although synchrotron radiation sources will soon be widely available in numerous countries, the technique still shows massive potential for further development. The Daresbury ring at full power produces several tens of kilowatts of raw radiation, enough to destroy most experimental specimens. Yet, because the requirement is mainly for spectrally pure radiation, most

of the power is dissipated as heat in a monochromator. If by some means the power available could be channelled into radiation of one wavelength a new and even more extraordinary generation of experiments would become possible, investigating nonlinear properties of matter. Perhaps the most radical proposal is to combine the power of synchrotron radiation with the coherence properties of a laser and produce the so-called free-electron-laser. It is gratifying to see UK scientists involved in all phases of the development and exploitation of synchrotron radiation.

# **Coding complications**

from a Correspondent

READERS will recollect that the celebrated wobble hypothesis of Crick defines the nature of the interaction between the codons of messenger RNA and the corresponding anticodons of tRNA. In short, the codons are read one at a time by tRNA molecules. These form three base pairs between the bases in their anticodon and those in the codon that it responds to. The first two base pairs formed by the codon are standard Watson-Crick A-U or G-C pairs but in the third position G-U pairs are allowed, as are A-I, G-I and U-I where the I residue is in the anticodon.

Although there is considerable evidence that this is the normal mode of codon-anticodon interaction in both eukarvotic and prokaryotic organisms, evidence has been accumulating that things are not always as simple as this. Thus in mitochondria, it would appear that a single base in the third position of the anticodon can 'read' each of the four bases which might occur in the third position of a codon, or to put the matter another way, only the first two bases of the codon are relevant. This state of affairs resembles the 'two out of three' mechanism proposed by Lagerkvist<sup>1</sup>. More evidence for such anomalies now appear in two papers in Nature and in two in Cell.

Munz et al. (see this issue of Nature, p. 187) have studied serine specific tRNA in Schizosaccharomyces pombe. This organism utilizes two isoaccepting tRNASer with anticodons IGA and U\*GA (U\*is a modified uridine) respectively. The one with IGA should be able to respond to the codons UCU, UCC and UCA and the other only to UCA. By means of some clever genetic manipulations Munz et al. were able to show, in essence, that mutants in which the U\*GA species is not synthesized are not viable. If the possibility that the missing tRNA had some other essential role to play is excluded, this would seem to imply that UCA codons were not being properly recognized. The anomaly appears when it is noted that the other serine specific isoacceptor with IGA for its anticodon should have been able to recognize this codon according to the rules of wobble. Apparently it does not. The significance of this *in vivo* result is increased when it is realized that the bulk of the evidence for A-I interactions is derived from *in vitro* experiments.

Meanwhile, Diamond, Dudock and Hatfield<sup>2</sup> have isolated and sequenced an unusual tRNA species from bovine liver. This accepts serine but has CmCA for its anticodon, an anticodon normally expected to recognize the tryptophan codon UGG. The anomaly appeared when it was found from in vitro binding experiments that the tRNA responded to UGA (which is normally the opal stop signal) but not to UGG or any of the serine codons. It was further shown that the tRNA could act as a suppressor of termination as the first result might suggest. In other words the Cm in the third position had 'read' A rather than G as demanded by the wobble hypothesis. This result for a eukaryote had been anticipated in the work of Hirsch3 who described a tRNATrp in E. coli which had an anticodon CCA but which responded to UGA codons. Both the eukaryotic and the prokaryotic tRNA molecules also had anomalous features in their sequences in the vicinity of their DiHU stems and the possibility remains that this feature is somehow related to their anomalous codon reading properties.

Another curious anomaly has been discussed by Bienz and Kubli (see this issue of *Nature* p 188). *Drosophila melanogaster* 

produces two major tRNATyr species one of which has the first position of its anticodon occupied by the modified Q base. The one with the unmodified anticodon, GUA, but not the other, is capable of acting as a suppressor to the amber termination codon UAG; in other words it can recognize UAG and insert tyrosine so preventing termination of translation. Again we have a wobble anomaly in that the interaction in the third position is between two G residues. The discovery of this naturally occurring suppressor in Drosophila was aided by an interesting assay system for breakthrough or suppression of a chain termination signal. This involved injecting into a Xenopus oocyte some of the tRNA to be assayed and some TMV RNA. If suppression occurred, this resulted in the formation of a 160,000 molecular weight protein, identifiable by polyacrylamide gel electrophoresis. Thus Bienz and Kubli also showed that wild type tRNATyr from yeast (with no Q base) would also act as suppressor in this system. They suppose that the presence of the Q base somehow inhibits the misreading of the UAG codons involved in this type of suppression.

Finally Bossi and Roth4 report on a different sort of suppressor effective in the suppression of the effect of a frameshift mutation. A frameshift mutant occurs when an extra base pair is inserted or a normal one left out during the process of replication. In either case the reading of the bases constituting the codons gets out of phase when the site of the mutation is encountered during translation. This results thereafter in a garbled polypeptide. Such a garbling of the message due to an insertion could be corrected if a tRNA was available which read four bases instead of three at a site near to the original mutation. Several mutant tRNA species can be produced which have this effect. Most of them respond to a region of the messenger RNA where there is a sequence of four identical bases; reading of the fourth base, so to speak, takes up the extra inserted base. The mechanism by which this occurs is obscure; maybe four base pairs are involved or maybe there is some sort of ambiguity as to which of the two possible triplets in the run of four the tRNA responds to. Bossi and Roth however, now describe a new suppressor, SufJ, which responds to sequences of four bases: ACCU, ACCC and ACCA (the sequence ACCG has not been tested). In other words it recognizes the triplet ACC but pulls the fourth base through the reading frame. This seems to be a sort of three out of four reading mechanism reminiscent of Lagerkvist's idea.

Simple explanation to account for these puzzling phenomena are not in sight but one thing seems certain: our once clear picture of the mechanism underlying the translation of the genetic message is developing a plethora of unbecoming flaws.

<sup>1.</sup> Lagerkvist Proc. natn. Acad. Sci. U.S.A. 75, 1759

<sup>2.</sup> Diamond, Dudock & Hatfield Cell 25, 497 (1981)

Hirsch J. molec. Biol. 58, 439 (1971).
 Bossi & Roth Cell 25, 489 (1981).

# Eight new Nobel laureates

# A spectrum of achievements

from David D. Burgess

One half of the Nobel Prize in Physics was awarded jointly to Nicolaas Bloembergen, Harvard University and Arthur L. Schawlow, Stanford University, for their contribution to the development of laser spectroscopy, and the other half to Professor Kai M. Siegbahn, Uppsala University for his contribution to the development of high-resolution electron spectroscopy.

SPECTROSCOPY, the study of all aspects of the interaction of electromagnetic radiation with matter in all its forms, not necessarily simply atomic or molecular, (undergraduate memories of thousands of atomic energy levels notwithstanding!), remains by its very nature one of the most powerful tools in large areas at the frontiers of both physics and chemistry. What is more interesting is that the prize has been given to this year's three Nobel laureates not for a specific scientific application, but for a more general and extended series of developments in technique. For obvious physical reasons the first demonstrations of methods of the type pioneered by the three Nobel winners have been in relatively 'pure' areas of atomic and molecular physics. However, the likely impact is much wider both scientifically and with important uses already developed in some technological and industrial spheres.

Schawlow and Bloembergen share half the prize for developments in laser spectroscopy. Some spectroscopic applications of lasers, of course, are simply extensions in kind of conventional techniques. Obvious increases in spectral resolution (from the very narrow bandwidths available), in signal (from high input intensities) or in time-resolution (from short pulse length) have been widely exploited. However, what Schawlow himself termed "The Laser Revolution in Spectroscopy" goes much deeper, stemming from two separate features which, in the visible and UV, occur only when laser sources are used. One is the ability to reach intensities at which the interaction of an atom or molecule with the electromagnetic field dominates that of all other interactions with its environment. whether radiative or collisional. The second is that the interaction of the atom or molecule with the electromagnetic field itself may become nonlinear.

Schawlow's achievements primarily relate to the first point. The power of spectroscopy becomes greatly enhanced if specific atoms or molecules in an ensemble can be 'tagged' in order to distinguish them from their surroundings, for example, by

selectively exciting only specific chemical or isotopic species, specific velocity subgroups, specific bound states, and so on. Almost the only way to do this is by interaction with laser radiation. A trivial calculation shows that at optical frequencies, thermal light sources, however bright and well-focused, cannot in general provide irradiances sufficient to cause significant selective excitation in the face of the always competing spontaneous radiative decay of the selected atomic or molecular state. Other excitation methods, for example, with pulsed electron beams, are not usually state selective. However, laser radiation provides both the intensity and the spectral resolution to change any selected state population in a sample at will, almost regardless of competing processes, and therefore to 'tag' chosen atoms.

When widely tunable lasers first became available in the late 1960s, Schawlow (a progenitor of the laser itself in his earlier work with Townes) left the then fashionable topic of laser development largely to others and with his group concentrated on a series of spectroscopic applications, often of a devastating simplicity and elegance. Prime amongst these was the development of 'Dopplerspectroscopy, by saturation spectroscopy, by 2-photon excitation, and by polarization spectroscopy, in which limitations on resolution always set previously by the thermal motions of atoms and molecules in a sample were removed either by selective excitation of particular velocity sub-groups, or in 2-photon spectroscopy by the actual cancellation of the Doppler shift regardless of the individual absorber's velocity.

The impact of the consequent vastlyincreased resolution on atomic and molecular physics is obvious, with a remeasurement of the Rydberg to increased precision by the Stanford group, a greatly increased ease of observing, for example, isotope shifts or high quantum states now exploited by many groups worldwide, and proposals even for looking at aspects of atomic physics related to current Unified Field Theories. Schawlow and his collaborators also demonstrated other possibilities of the immense sensitivity of laser spectroscopy, for example the capability of detecting just one atom of a given species against a background of other

species of almost arbitrary density, at one step not only pushing back the boundaries of chemical trace analysis by several orders of magnitude but indeed extending them to their ultimate physical limit. More recently, Schawlow has developed spectroscopic methods simplifying the analysis of molecular structure by selectively laser-tagging specific states.

Many of these spectroscopic developments have applications far outside pure atomic and molecular physics. The most obvious case is Doppler-free spectroscopy which is very intimately related with some schemes for obtaining efficient laser separation of fissile isotopes. However, many of the applications of the vast increase in spectroscopic sensitivity remain to be explored. (A single but rather nice example, given in a review by Letokhov at the recent Heidelberg European Atomic Physics meeting, is that the enormous sensitivity of laser spectroscopy for measuring relative isotopic abundance may now render all other radiocarbon and related dating methods quite obsolete.)

Bloembergen's work includes both theory and experiment in areas very similar to those described above, but perhaps best known is his contribution to the analysis of the possible nonlinear interactions of an atom or molecule with the electromagnetic field itself when the field intensity is high. The number of such nonlinear processes is vast. As well as fundamental interest many already have technological applications, whether to parametric losses in optical communication systems, spectroscopic analysis of jet engine exhausts via CARS (coherent anti-Stokes Raman Spectroscopy), atmospheric self-focusing of high-intensity laser beams due to nonlinear refractive indices, or recent developments of 'phase-conjugate' reflectors opening the possibility of optical systems in which aberrations and defects, however severe, are automatically self-cancelling. Important spectroscopic applications result from the availability of frequency up- or down-converted coherent sources in difficult spectral regions such as the vacuum ultraviolet and also from the more direct use of related '4-wave mixing' processes such as CARS and its derivatives as immensely sensitive spectroscopic tools.

The theoretical prescription provided by Bloembergen and his group, notably in a crucial paper in 1962, provided a suitable framework for distinguishing between and analysing the many possible competing nonlinear effects, particularly processes leading to frequency up or down

David D. Burgess is Professor of Spectroscopy at The Blackett Laboratory, Imperial College of Science and Technology, London. conversion, laying an immensely timely foundation for the type of applications already mentioned. Lately, Bloembergen has also become interested in a further technological possibility of selective laser spectroscopy of molecules, that of laser-induced chemistry, or laser catalysis, in which the direction and speed of a chemical reaction may be controlled by selective excitation of specific states in the reactants, or by selective destruction of the reactant products.

Laser spectroscopy has taken a large share of the fashionable limelight in recent years, but the award of half of the prize to Professor Kai Siegbhan is a reminder of equal recent progress in areas where lasers are not yet available, particularly the VUV and X-ray regions. Siegbahn's achievements relate to specific uses of photoelectron spectroscopy, the accurate measurement of the energies of electrons ejected by atoms or molecules irradiated with VUV or X-ray wavelengths. Siegbhan's contribution has been ESCA. Electron Spectroscopy for Chemical Analysis. In the complex spectra of most molecules, the structure of the individual atomic valence states is so perturbed by the bonding process that even in simple cases the visible or ultraviolet spectrum is not usually an a priori guide to the atomic constituents. Siegbahn realized that this could be overcome at X-ray wavelengths. the inner atomic electrons being largely shielded from the molecular environment, and the excitation energies therefore remaining characteristic of the atomic species involved. However, with laser techniques yet unavailable at X-ray wavelengths, orthodox emission or absorption spectroscopy does not provide a full basis for such an analytic method, emission requiring temperatures frequently sufficient to destroy the molecule of interest, and absorption work often needing excessively large samples. Siegbahn, therefore, used the alternative technique of energy analysing electrons ejected from the inner shells of atoms in molecules irradiated with specific X-ray wavelengths, a technique applicable to relatively small samples. The spectra so obtained additionally allow the investigation of the particular bonding environment of each atomic species, as small 'chemical shift' of the inner-shell binding energies by outer bonding electrons remains sufficient for aspects of the bond to be characterized without loss of the atomic identity of the spectra needed for direct chemical analysis.

In the past fifteen years spectroscopy has ceased to be simply a powerful but established technique, in some ways changing technically more than at any time since Newton. A huge challenge remains in both pure and applied science in the yet untapped applications of the type of techniques developed by the three Nobel winners in what now almost constitutes a completely new subject.

# Hemispheric modes of consciousness in the human brain

from Colwyn Trevarthen

One half of the Nobel Prize in Physiology or Medicine was awarded to Roger W. Sperry, California Institute of Technology, for his discoveries concerning the functional specialization of the cerebral hemispheres.

ROGER SPERRY, a leader among students of the functional design of the brain for forty years, might well have received the Nobel Prize for experiments he did in the 40's on the self-sorting of nerve pathways regrowing after surgical rearrangements in fish and amphibia. Many neurobiologists still see that as his greatest work.

The split-brain studies for which he received the prize last month led to discoveries about the human brain which are, however, of far greater importance for psychology. They showed that each human cerebral hemisphere, when disconnected from the other by surgery, could formulate consciousness, feelings and intentions on its own and that left and right sides had different mental abilities.

The experiments with human subjects came directly out of micro-surgical research at the University of Chicago on the transfer of learning from one side of the brain to the other in fish. When Sperry and Ronald Myers applied this approach to cats in the 1950's, they achieved the first convincing surgical duplication of the brain systems serving memory formation and awareness in one animal. Their brilliant, microscopically controlled operations sliced through the cross-over of visual input pathways under the brain, and the corpus callosum between the cerebral hemispheres.

Sperry's studies of split-brain cats and monkeys continued at the Californian Institute of Technology after he became Hixon Professor of Psychobiology there in 1954.

Then, in the 1960's, the elegant behavioural tests he had already devised for animals showed Sperry the way to effective study of human commissurotomy patients. This gave the breakthrough to clearer understanding of specifically human states of mind.

A Los Angeles neurosurgeon, Joseph Bogen, observing the rapid recovery of monkeys after Sperry's split-brain surgery, recalled that the corpus callosum of a human being could be transected safely in selected patients with uncontrollable epilepsy, to interrupt the seizures that were devstating these peoples' lives. A small

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group of patients were operated upon by Philip Vogel and Bogen during the 60's producing, a successful alleviation of their epilepsy and a marked improvement in their health. Sperry worked with Bogen and a number of graduate students, of whom Michael Gazzaniga was the first, in the discovery of a most extraordinary division of human consciousness in these persons: a duplicate state of mind largely hidden when the patients were in free mastery of their experience and actions. Sperry led a systematic exploration of the full scope of this effect and so clarified a uniquely human division of mental tasks between the left and right halves of the

That parts of the left hemisphere are dominant in the control of speaking and writing and for understanding language was discovered in the last century. Observation of the psychological effects of wounds in different parts of the cortex produced evidence for each and all of the hemisphere differences that Sperry disclosed in his investigation of commissurotomy patients. But the history of this quest did not allow a coherent overview. There had never been a brain chart like Sperry's which summarized the basic division of functions, giving equal attention to both hemispheres. Even though a group of psychologists had been saying since the 40's that the right hemisphere could be superior to the left in certain non-verbal visuo-spatial kinds of detection and reasoning, their point was not well-taken by the majority of neurologists who were inclined to identify human intelligence with verbal intelligence.

The tests devised by Sperry's group showed that an isolated left hemisphere had clear superiority for functions linking a sequential pattern of logic with the production of speech and writing. They also showed that right hemisphere consciousness was not a weak copy of the left. It could perform some tasks better than the left even though it could not use elaborate verbal imagery or rational thinking of the propositional variety. Thus, the tests clarified our intuitive nonverbal insight and concrete awareness and drew attention to the work the brain must

Accounts by R.W. Sperry of his work are found in Cerebral Organization and Behaviour, Science 133, 1749 (1961); Mental unity following surgical disconnection of the cerebral hemispheres, Harvey Lectures 62, 293 (1967); Mind-Brain Interaction: Mentalism, Yes: Dualism, No. Neuroscience 5, 195 (1980); Changing Priorities, Annual Review of Neuroscience 4, 1 (1981).

do to directly percive and understand persons and things in the humanly ordered world about us. The right hemisphere was shown, for example, to be strongly preferred for feeling strange shapes in the hand, for copying drawings and for recognizing faces.

Sperry and his students, notably Jerry Levy, Robert Nebes, Harold Gordon, Eran and Dahlia Zaidel and the writer, adapted psychological tests to show how a splitbrain person could be conscious with one halfof the cerebral cortex at a time, or how he or she might choose to distribute a given cognitive task between the two disconnected halves. In all this work, which has produced many renowned scientific papers, Sperry acted as navigator and critic, employing his remarkable literary skills to draw the pieces of the puzzle into a bold harmony of primary principles.

Sperry has written important papers on the philosophical implications of splitbrain research, regarding the casual potency of consciousness as a spatiotemporal brain process that creates reality, goals, values, knowledge and skills in brain organs with a definite inherited anatomical design. Cerebral anatomists are now actively seeking inborn differences in the organization of cortical tissue in the two hemispheres. Psychology is encouraged to look inside the mind to understand internal and preordained standards of 'will', 'desire', 'imagination' and 'understanding' that condition how experience will affect our actions.

The studies of split-brain patients have opened the way, also, to a developmental approach or to an embryology of the mind. Human cognitive systems grow over many decades. Interaction of the hemispheres while they develop in the fetus and through childhood results in a segregation of major functional systems in a partnership or federation. Power sharing is thereby achieved with a much higher efficiency in the integration of an individual's purposes and adaptive actions. This process takes a different course in right and left handers, in males and females, educated and uneducated, the blind and the deaf. There takes place in each of us a progressive division of brain work and generation of a unique bihemisphere condition. Genetically based hemispheric specialisations enter into regulation of the individual's place in his family, his society, his culture and in the history of his community.

been known that the contralateral eye sends fibres to laminae 1, 4 and 6 in the nucleus which relays information to the visual cortex, with laminae 2,3 and 5 supplied by the ipsilateral eye. Hubel and Wiesel made electrolytic lessions limited to a single lamina in the relay nucleus and found degenerating nerve endings in a series of regularly spaced bands about  $400 \mu$  wide and separated by  $400 \mu$  throughout the visual cortex. If their lesion in the relay nucleus encroached on an adjacent lamina, the cortical degeneration became continuous.

The existence of right and left eye ocular dominance columns was fully confirmed by other methods, and they have been found in cats, galago and the macaque, but not in the mouse, the tree shrew or the squirrel monkey. The basic unit seems to be an about one millimetre square 'hypercolumn', containing left and right ocular dominance columns crossed approximately at right angles by about ten orientation columns.

It was previously known that retinal fibres project on the visual cortex in an orderly fashion, so that in man the fovea, the point of most distinct vision, is represented at the occipital pole and the extreme periphery at the front end of the calcarine fissure, and it was known from Gordon Holmes's work that the fovea had a disproportionately large area of representation in the cortex. It became clear that a hypercolumn of one square millimetre in the foveal area would deal with a solid angle of 10 minutes of arc, whereas a similar hypercolumn in the far periphery would cover about 10 degrees. There is a continuous representation of the visual field along layer IV in each ocular dominance column, half overlapped by the representation in the adjacent column for the other eye. In this way topographical representation, provenance from each eye and orientation are all represented in a two dimensional sheet of cortex.

Hubel and Wiesel have also established that ocular dominance and orientation preference is present at birth in cats and monkeys without visual experience. Newborn animals differ from adults in having a less sharp tuning of orientation preferences and less binocular interaction. These differences seem to be due to lack of visual experience rather than immaturity as animals whose eyes are kept closed for some weeks from birth show little improvement on the state at birth. The system is still plastic, however, since closure of one eye soon after birth results in the take-over of most of the cells of layer IV by afferent fibres from the open eye so that after some weeks the deprived eye drives very few cells in the cortex. This plasticity lasts for about three months in kittens, somewhat longer in monkeys and up to one to two years in man. Closure of one eye after this 'critical period' has no permanent effect and reopening and retraining must take place during the critical period if there

### Visual machinery of the brain

from David Whitteridge

One half of the Nobel Prize in Physiology and Medicine was awarded jointly to David H. Hubel and Torsten N. Wiesel, both of the Harvard Medical School, for their discoveries concerning information processing in the visual system.

THE work of Hubel and Wiesel over the last twenty years has established two main points: first, that the visual cortex has a remarkably ordered fine structure, composed, in the monkey, of about a thousand units of identical structure, each of which contains mechanisms for the analysis of all the data from corresponding parts of the visual fields and, second, that these orderly units are very largely established by genetic mechanisms during prenatal development and can be modified by visual experience only during the first few months of life.

Their first major discovery was that cortical visual cells are excited by bars, light or dark, or by single edges, and that each cell responds optimally to a particular orientation of the stimulus. This was previously unsuspected, as ganglion cells of the retina and the cells of the lateral

geniculate (relay) nucleus were known to have round fields, usually concentrically arranged so that an excitatory centre would be surrounded by an inhibitory surround and vice versa.

To analyse all possible patterns it would be necessary to have sets of cells sensitive to a complete range of orientations. In the cortex such an organization has been found. Cells of one orientation occupy a "column" about 50 wide, extending from the surface to the white matter, with columns of approximately ten degrees different orientation, displaced clockwise or anticlockwise, on each side. A horizontal penetration through the cortex encounters a regular series of such orientation columns occasionally interupted by reversals. Much later, Hubel and Wiesel were able to display the full extent of orientation columns by injecting radioactive deoxyglucose, and exposing the animal to light bars at a single orientation. A series of regularly spaced thin curved columns were found to be stained.

They established a second independent set of 'ocular dominance' columns each of which received fibres from one eye only and contained cells which were driven exclusively (in cortical layer IV) or predominantly by that eye. It had long

David Whitteridge is Emeritus Professor of Physiology in the University of Oxford. is to be any recovery. The mechanism by which active fibres can apparently displace inactive fibres is obviously of great importance and as yet not understood.

This knowledge of the organisation of

the primary visual cortex is being used to understand the activities of other visual areas and is forcing reconsideration of present ideas on the *modus operandi* of all other cortical areas.

### Rules for chemical reactions

from Graham Richards

One half of the Nobel Prize in Chemistry was awarded to *Kenichi Fukui*, Kyoto University and the other half to *Roald Hoffmann*, Cornell University, for their theories concerning the course of chemical reactions.

THEORETICAL CHEMISTS often seem to work at one of two extreme ends of the same problem. Either they do highly accurate calculations on small molecules or crude, even qualitative, investigations of larger molecules which are closer to the molecules synthesized and used at the laboratory bench. Both Fukui and Hoffmann belong to the latter school. Their work has added an excitement to broad areas of organic chemistry. The culmination of their research in the form of Woodward-Hoffmann rules represents what is perhaps the most significant single step in organic chemistry in the post-war period.

The chemistry of a molecule is determined by the distribution of its electrons and by how tightly these electrons are held at particular locations in the molecule. Fukui's method of frontier molecular orbitals concentrates on the electrons which are the most loosely bound. If a reactant is involved in donating electrons to form bonds then the electrons

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involved are most likely to be those most easily removed. Conversely, if a molecule reacts by virtue of acceptance of electrons into a previously unoccupied molecular orbital then the bond will be created in the region where the extra charge is most readily accepted. Frontier molecular orbital theory then looks quantitatively at electron density distributions in the occupied molecular orbital which is highest in energy terms and at the lowest unoccupied molecular orbital. The new acronyms HOMO and LUMO abound in current literature on organic and medicinal chemistry.

Frontier orbital theory is based on the perturbation treatment of molecular orbital theory introduced by Coulson and Longuet-Higgins. It deduces conclusions about chemical reactions by looking at the interaction of the reactants, assuming that the position of the transition state on the potential energy surface can be deduced from the initial slope along the reaction coordinate; the smallest slope is identified with the lowest energy transition state and consequently the fastest reaction or preponderance of products. Fukui's ideas were developed and exploited in the early days when theoreticians were not blessed with large computers and in consequence restricted to pi-electron systems and the simple Hückel molecular orbital method. This limitation nonetheless allows consideration of the reactivities of all the aromatic molecules of orgnic chemistry

since their reactions are largely those of the delocalised pi-electron system built from atomic 2p atomic orbitals.

Hoffmann's first major contribution to theoretical chemistry was to extend the formalism of the pi-electron Hückel theory to include sigma electrons. Thus molecules of the aliphatic type such as ethane could be considered as well as aromatics such as benzene. This simple semi-empirical molecular orbital technique is still widely used since it can treat very large molecular systems without demanding anti-social amounts of computer time.

Experience gained from extended Hückel calculations led to the now famous Woodward-Hoffmann rules which Fukui showed could be explained by frontier orbital theory, (although there are other explanations including symmetry arguments). The idea of Hoffmann and the late Nobel laureate R.B. Woodward explains why, for example, maleic anhydride reacts easily with butadiene but not at all easily with ethylene. In the former case two new carbon-carbon bonds are made simultaneously and the electrons which are involved complete a circuit. Concerted and cyclic reactions of this type are called pericyclic and this example is a cycloaddition. Woodward and Hoffmann's rules start with a consideration of the molecular orbitals of the reactants and match up the electron densities in occupied and unoccupied molecular orbitals. A correlation diagram permits a simple decision to be made as to whether a particular reaction is allowed or not.

Vast numbers of applications of the Woodward-Hoffmann rules are to be found in recent literature and perhaps the best testament to the power and range of applicability of these rules is to quote in its entirety the first paragraph of chapter 12 from the eponymous pair's book *Conservation of Orbital Symmetry*; Chapter 12, Violations "There are none!"

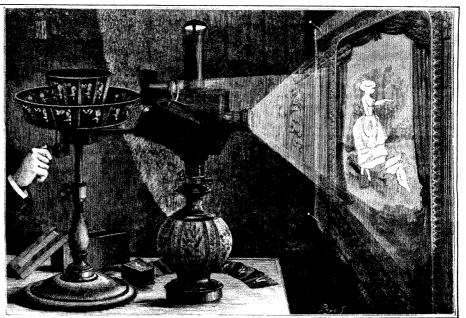


### 99 Years ago

THE PROJECTION PRAXINOSCOPE

M. Gaston Tissandier describes in La Nature an ingenious adaptation of the praxinoscope by means of which the images are projected on a screen, and are visible to a large assembly. M. Reynaud, the inventor obtains at once the projection of the scene or background - by the object-glass which is seen at the side of the - and of the subject, by another object-glass which is shown in front of and a little above the same lantern. In making the two parts of the apparatus converge slightly, the animated subject is brought into the middle of the background, where it then appears to gambol. A hand-lever on the foot of the instrument allows a moderate and regular rotation to be communicated.

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## REVIEW ARTICLE

### A new era in mammalian gene mapping: somatic cell genetics and recombinant DNA methodologies

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Mammalian gene mapping techniques are now sufficiently advanced to contribute significantly to prenatal diagnosis and to human molecular genetics. Restriction fragment mapping can be used to place polymorphic genetic markers at random sites within the genome, and these sites used to assign genes responsible for disease conditions to a chromosomal region. Somatic cell genetic techniques can then be applied to saturate that region with additional restriction fragment markers, some of which will be closely linked to the disease gene. Closely linked restriction fragment markers, especially flanking pairs of markers, can act as predictors for the transmission of defective genes to offspring. A series of tightly linked flanking restriction markers might in addition contribute to the eventual isolation and cloning of the disease gene itself.

SOMATIC cell genetics provides a means of analysing the complex genetic organization and regulation of higher eukaryotes<sup>1,2</sup>. Although it has contributed significantly to our knowledge of mutagenesis3, genetic complementation4 and gene regulation<sup>5</sup>, its major success has been in gene mapping, especially in man<sup>6,7</sup>. Two approaches to the analysis of the human genome have been remarkably successful. Recombinant DNA techniques have allowed several genes and their flanking DNA regions to be sequenced, and using such techniques, functional regions, such as promoters for RNA synthesis, have been identified and mutations and deletions precisely defined. At the level of the whole chromosome somatic cell genetic techniques have been used to map hundreds of human genes directly, and hundreds more have been mapped on the basis of known linkages to the first group. Until recently analysis of the intermediate level of human genetic analysis has been much less successful. Now, however, a variety of approaches make this range accessible as well. This review focuses on these approaches and on the way in which their concerted application will yield both a better understanding of eukaryotic genome structure, and information useful in clinical diagnosis.

### Gene transfer between mammalian cells

Gene mapping by somatic cell genetics depends primarily on parasexual events which facilitate the transfer of genetic material between cells (gene transfer) and the loss of transferred material from hybrid cells (segregation). In discussing gene transfer systems, it is convenient to distinguish between the donor cell which serves as the source of transferred genetic materials and the recipient cell into which donor genetic material is delivered. The resulting heterogenote is referred to simply as the hybrid cell. There are four ways of delivering genes into recipient cells:

(1) Cell hybridization: Fusion of two genetically distinct parental cells (for example, man×mouse) yields cell hybrids which contain initially complete parental chromosome sets. In such interspecific hybrids, one chromosome set is usually partially lost (segregation), and for convenience this set is termed the donor set. Hybridization thereby provides a means of transferring large numbers of donor chromosomes into a recipient cell. Hybrid clones can be established which retain a defined set of donor chromosomes.

- (2) Microcell-mediated gene transfer: Donor cells can be manipulated so that their chromosomes become isolated in microcells<sup>8</sup>, which may possess only one or a few donor chromosomes. Such cells can be hybridized to recipient cells, and thereby serve as convenient vectors for the transfer of one or a few intact donor chromosomes into recipient cells. An advantage of the microcell approach is experimental control over chromosome segregation.
- (3) Chromosome-mediated gene transfer: Cell-free preparations of donor chromosomes can be used to transform recipient cells<sup>9-10</sup>. In this system, whole chromosomes are taken up by endocytosis and then degraded into subchromosomal fragments. Fragments large enough to be detected by light microscopy as well as submicroscopic fragments have been described<sup>11</sup>. The lower limits of fragment size have not yet been
- (4) DNA-mediated gene transfer: Eukaryotic cells can be transformed genetically by purified DNA<sup>12</sup>. Two primary means of transfer exist: endocytotic uptake of calcium phosphate precipitated DNA, and direct injection of purified DNA into the nucleus by microcapillary pipettes. Transformation frequencies obtained by microinjection can be as high as 20% (ref. 13), whereas transformation by endocytosis of precipitated DNA (or the endocytotic uptake of chromosomes) is generally four to five orders of magnitude less efficient. In DNA-mediated transfer, the size of the donor fragment initially taken up by the recipient cell is invariably small, usually less than 50 kilobases (kb).

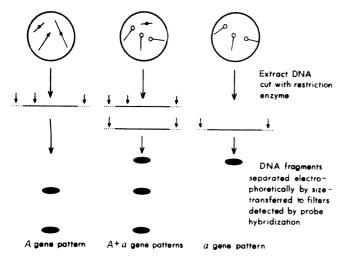
Thus it can be seen that through the choice of the particular gene transfer system used one can control within broad limits the amount and size of donor material transferred into a recipient cell. As we shall see below, this capability provides a means of regulating the resolution of gene mapping analysis.

### Gene mapping by somatic cell genetics

The assignment of genes to chromosomes and regions of chromosomes in somatic cell genetic systems is accomplished by correlating a particular donor gene or its product with a specific donor chromosome or a subchromosomal fragment <sup>14</sup>. More than 200 human genes have been assigned to particular chromosomal locations in the human genome by this method, bringing the total number of mapped genes in man to more than 400. On the average, four human genes are mapped per month

by the somatic cell approach, and as we shall see, this accession rate can be expected to increase dramatically in the next few years.

Until recently, the method of choice in gene assignment was the correlation of a particular gene product (such as an enzyme, structural protein or surface antigen) with an identifiable donor chromosome or fragment. This method has severe limitations, because it depends on the expression of the donor gene and thus precludes the mapping of genes which are expressed only in certain differentiated cells or of nucleotide sequences which have no coding functions. These drawbacks can be overcome by using nucleic acid hybridization techniques to detect donor genes directly. We first showed the feasibility of this approach in the assignment of the human  $\alpha$ - and  $\beta$ -globin loci using solution hybridization ( $C_0t$  analysis) of  $\alpha$ - and  $\beta$ -globin cDNAs with DNA preparations from hybrid clones containing different human chromosomes. These studies showed that the  $\alpha$ -globin complex mapped 15 to human chromosome 16, and the  $\beta$ -globin complex mapped<sup>16</sup> to human chromosome 11. The success of this method depends on the specificity of hybridization of the donor gene probe with the homologous donor human genomic sequence, and so appreciable cross-reaction with recipient cell DNA sequences will limit the use of solution hybridizaton as a gene mapping technique. This represents a serious restriction, because in many instances cross-hybridization of structural gene probes can be expected to occur, as we found to be the case when we attempted to extend the solution hybridization procedure to the assignment of the murine immunoglobulin genes. In this system, there was extensive cross-hybridization between cloned recombinant DNA probes of specific mouse immunoglobulin nucleotide sequences and the recipient (in this case, Chinese hamster) genomic sequences. This problem was solved by making use of donor and recipient differences in restriction endonuclease sites within or around the gene of interest and using the Southern blotting procedure to reveal these differences (Fig. 1). The differences in the location of restriction sites are diagnostic for the donor and recipient forms of a particular immunoglobulin gene. Using this approach, it was possible to assign the mouse  $\kappa$ ,  $\lambda$  and heavy chain complexes to chromosomes 617, 1618 and 1219 respectively. The restriction fragment mapping procedure has the advantage that it is independent of hybridization specificity, depending only on the restriction fragment length differences which occur frequently between species. This approach makes possible the mapping of any unique or low copy number nucleotide sequence, and within



Chromosome 1 correlates with the A gene pattern  $\therefore$  the A gene can be assigned to chromosome 1

Fig. 1 Restriction fragment mapping. The restriction pattern of the donor cell gene (A) differs from that of the recipient cell gene (a). The probable region of the genes is depicted by solid lines. Diagnostic fragments of different molecular weight are generated from the two genes by endonuclease digestion. The signature of the donor gene can be correlated with a particular donor chromosome.

a relatively short period, an impressive number of gene complexes have already been assigned in human and murine genomes using this procedure (Table 1). As the number of cloned DNA probes to specific genes is expanding rapidly, we can expect the mapping of such genes to increase commensurately.

Chromosome cloning techniques (that is, the isolation of single donor chromosomes in a host recipient cell) can also be used to assign a gene to small subregions on a particular chromosome and to establish the order of different genes along the length of the chromosome<sup>20</sup>. This can be accomplished by using donor cells which carry reciprocal translocations involving a particular chromosome. Such translocations occur at a relatively high frequency in human families where they are detected as a consequence of high abortion rates or congenital defects. A concerted effort has been made to collect cells from translocation carriers<sup>21</sup>; more than 300 such translocations have been stored, and for each the translocation breakpoints have been described in terms of chromosome banding patterns. If a gene has been assigned to a particular chromosome, then a series of translocations involving different sites along the chromosome can be used to delimit the position of the gene. This position has been termed the 'smallest region of overlap' (SRO)22. Translocations or deletions which arise spontaneously in the hybrid cells can also be used for this purpose. More than 100 human genes have been assigned to subchromosomal regions by these methods. Such regional mapping procedures lend themselves readily to restriction fragment mapping; for example, the human  $\beta$ -globin locus was assigned to the chromosomal subregion 11p125-p128 in this way

Human reciprocal translocations can also be used in conjunction with chromosome sorting techniques to fix the subchromosomal localization of genes. It has been shown that isolated mammalian mitotic chromosomes can be sorted into discrete size groups by fluorescence particle sorting<sup>24</sup>. Nearly every human chromosome can be resolved as a unique size class by this technique. DNA can be isolated from the sorted chromosomes and then hybridized with cloned probes in order to establish gene assignments. Translocations are particularly useful in this mapping procedure because they alter the size of a chromosome in a predictable way, causing a change in the sorting pattern, and thus enabling a suspected linkage relationship to be verified25. Drawbacks of the chromosome sorting approach involve the relatively long sorting times required for recovery of sufficiently large samples and the high cost of the procedure. Despite these limitations the technique can be expected to add another dimension to gene mapping methodologies.

Considering all the regional mapping procedures, probably the greatest potential lies with in situ hybridization26. This technique is used routinely to locate single copy genes in Drosophila polytene chromosomes, where they are amplified about 1,000 times. The older in situ hybridization procedures could reliably detect single copy genes amplified as little as 100-fold-but not less. Recently new procedures have been introduced which significantly increase the sensitivity and resolution of the method so that a single copy gene amplified 10-20-fold can be detected<sup>27</sup>. It may also be possible to detect unamplified unit genes using additional refinements; in fact, several investigators have claimed success in mapping single copy genes by *in situ* hybridization<sup>28-31</sup>. Recent innovations include: the use of <sup>125</sup>I-labelled nucleotide, introduced into DNA probes by nick translation, which produces radioactive probes of high specific activity (up to 10° d.p.m. per µg DNA)<sup>27</sup>; the use of dextran sulphate in the hybridization reaction mixture to increase hybridization efficiency<sup>32</sup>; single-stranded probes which do not hybridize to themselves, thus providing an additional increased efficiency of approximately five- to sevenfold<sup>33</sup>. More recently, it has been shown that biotin derivatives of cytidine can be produced (D. Ward, personal communication), which can be efficiently incorporated into probes by nick translation. DNA containing biotinized nucleotides can

Table 1 Recent progress in restriction fragment mapping

Human			Mouse			
Chromosome position	Gene	Ref.	Chromosome position	Gene	Ref	
2-	Proopiomelanocortin	54	3	r-protein L30	62	
2p	Prolactin	55	5	r-protein S16	62	
6 9 "	$\alpha$ - and $\beta_1$ -interferons	56	5	J protein	63	
9 11	Insulin	57	5	Casein complex	67	
	β-Globin complex	23	5	α-Fetoprotein	65	
11p125-p128	Four anonymous restriction	<b>4</b> -2	6	r-protein L19	62	
11p11-p13 11p128-p11 11p13-p128	fragments	50	6	к Light chain immunoglobulin complex	17	
11p13-pter			7	r-protein L18	62	
17.01	Growth hormone complex	58, 59	11	α-Globin complex	66	
17q21-qter	Unknown restriction fragments	61	12	Heavy chain immunoglobulin complex	19	
X	Olikilowii restriction fragments	0.	12	r-protein L18	62	
			12	r-protein L30	62	
			16	λ Light chain immunoglobulin complex	18	
			17	$\alpha$ -Globin pseudogene $\psi$ 3	66	
			18	$\alpha$ -Globin pseudogene $\psi$ 4	66	

be used as antigens to produce specific polyclonal or monoclonal antibiotin DNA antibodies. Such antibodies can be fluorescently, isotopically or enzymatically labelled, and then used as a second label against the polynucleotide probe. Labelled antibodies directed against the antibiotin DNA antibodies can also be produced and used to increase signal intensity.

Theoretically, combinations of the above techniques should permit the regional localization of unit genes. The in situ method is all the more promising when one considers that many gene loci exist as complexes containing clusters of related genes. If mixtures of the relevant probes were used, one would expect a corresponding increase in sensitivity. It may also be possible to use the in situ technique in combination with restriction fragment mapping of chromosomes using cell hybrids. For example, a cloned probe could be used initially to map a gene to a particular chromosome using the restriction fragment mapping technique, and then the same probe could be used in situ to localize the gene to a specific region on the chromosome. The advantage of the sequential procedure is that only one chromosome need be considered in the in situ analysis, thus reducing the possibility of error due to nonspecific binding of the probe to irrelevant chromosomes. Such an approach should improve the signal to noise ratio by 20-fold.

### Resolution power of mapping techniques

The resolution achievable by the subchromosomal mapping procedures described above lies in the range of 5–10 centimorgans. This estimate is based on the following considerations. Modern methods of chromosome banding permit the resolution of 850 bands per haploid genome<sup>34</sup>. Refinement of these methods can be expected to increase the band number to somewhat more than 1,000 (ref. 35). The total length of the human genome calculated in recombination units is about 3,000 centimorgans (ref. 36). Therefore, the number of bands is roughly one-third the number of centimorgans. Also relevant to the following discussion is the fact that the total human haploid nucleotide base pair number is about  $3 \times 10^9$ , and therefore there are an average of  $10^6$  base pairs (1 Mbp) per centimorgan.

Conventional low-resolution somatic cell genetic procedures can be expected to map genes to chromosomes and then within chromosomes to a resolution of 5–10 centimorgans. At the other end of the scale, high-resolution recombinant DNA procedures together with restriction site and nucleotide sequence analysis can be expected to provide mapping data from the level of the single nucleotide base pairs to the 100-kb level. These distances can be spanned by 'walking' along a chromosome from an identified cloned genomic DNA fragment using recombinant DNA techniques. This method involves isolating a DNA fragment within a genetically defined region by recombinant DNA

cloning from which the walk is started. This fragment is then used to probe a genomic library to identify clones which share a region of homology, but which extend beyond the initial fragment. These clones are then isolated, the process repeated and the linear nucleotide sequence extended. The 'walk' method combined with detailed cytogenetic maps has been used to advantage in *Drosophila*. However, *Drosophila* is especially well suited to this approach because there are relatively few redundant sequences which introduce false branch points in the walk, and because of the availability of the polytene in situ hybridization technique which provides a means of verifying progress along the chromosome (W. Bender, personal communication). However, mammalian genomes do not have these advantages.

Thus, in mammalian genetic linkage analysis a resolution 'gap' in the gene mapping techniques exists over the range 0.1-5.0 centimorgans (0.1-5.0 Mbp) (Fig. 2). This is a significant gap, because many functionally related clusters of genes, such as the human major histocompatibility locus, will fit into a space of this dimension. What mapping strategies might be devised to fill this gap?

Gene transfer systems provide possible approaches to intermediate level resolution analysis. All these methods depend on fragmenting linkage groups into small bits. In addition, all require a selectable marker in the linkage group of interest. The frequency of recovery of genes in linkage with the selectable marker provides information on the distance and order of genes with respect to the selectable marker. However, the requirement for a selectable marker has become less of a problem with the advent of DNA-mediated gene transfer. It is now possible to integrate various selectable markers into any linkage group by this procedure.

The three principal gene transfer systems suitable for intermediate level resolution analysis are: (1) DNA-mediated gene transfer, (2) donor chromosome breakage by irradiation and subsequent transfer to recipient cells by hybrid formation and (3) transfer of isolated metaphase chromosomes. The first system offers little advantage over walking, as the maximum fragment size is ~50 kb. The second method has already been successfully applied to linkage analysis by Goss and Harris37, who estimated the distance between the human genes thymidine kinase and galactose kinase to be 1.2 centimorgans by this technique<sup>38</sup>. The third method appears to have considerable potential for intermediate level resolution analysis, because a broad range of fragment sizes are generated by this procedure1 It has been shown that the procedure separates thymidine kinase and galactose kinase genes in 80% of transformants<sup>39</sup>, suggesting a potential for resolving genes separated by distances of 0.1 to possibly 0.05, centimorgans.

Klobutcher and Ruddle have recently introduced a breakagetranslocation based approach to middle-resolution gene

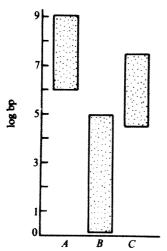


Fig. 2 Genetic mapping resolution by different methods. a, Gene mapping by somatic cell genetics; b, molecular genetic techniques; c, gene transfer and breakage-translocation methods. Ordinate = logarithm of base pair number.

mapping<sup>40</sup>. In this system, a donor chromosome is transferred into a recipient cell by one of the gene transfer techniques. The chromosome must carry a selectable marker which confers viability on the recipient cell when grown in selective conditions. An example would be the transfer of the thymidine kinase chromosome into a thymidine kinase-deficient recipient cell grown under hypoxanthine/aminopterin/thymidine selection. A second requirement is that the donor chromosome be lost (segregated) from the recipient cell at a frequency of  $\sim 3-10\%$  of cells per generation. A translocation between the donor chromosome and a recipient chromosome can give rise to an interspecific recombinant chromosome which is stably retained, and which carries the donor selectable gene and the recipient centromere (Fig. 3). Even though such translocation events are rare, the resulting hybrid cell possesses a 3-10% per generation growth rate advantage, and will rapidly come to constitute an appreciable fraction of the original population.

Independently arising recombinants of this type can be isolated and then the order and relative distances between genes in the chromosome segment lying between the selectable marker and the centromere can be analysed. In the first application of this approach, recombinants were obtained in which the human selectable marker thymidine kinase and the closely linked human constitutive marker galactonkinase were separated with a dissociation frequency of ~40% which indicates that resolution at the 0.1 centimorgan level can be achieved. The breaktranslocation system has several advantages: (1) a single well characterized cell line is used which continuously generates recombinant sports; (2) chromosome breaks are selected only within a well defined region determined by the donor centromere and the prototrophic, donor marker; (3) in primate/rodent combinations, appropriate chromosome staining techniques can be used which permit the detection of the chromosome break points and allow a cytological estimation of the size of the translocated donor fragment.

Thus, it can be seen that a variety of gene transfer procedures have potential for establishing the order of and distance between genes within the intermediate range of resolution. These techniques can be subjected to rigorous evaluation only when numerous unique nucleic acid probes have been isolated which map into appropriately short segments of mammalian genomes amenable to analysis.

## Cooperative interaction between mendelian and somatic cell genetics

In 1911 the first sex-linked gene (colour blindness) was mapped in man<sup>41</sup>. It was not until 1970 that the first human autosomal gene was mapped using a strictly mendelian approach. However, since 1970, around 50 additional autosomal genes

have been mapped in man using family analysis. This significant increase in the accession of autosomal genes controlling complex traits can be attributed in many cases to the previous chromosome assignment of genes by somatic cell genetics. The Rh gene, first identified in 1939<sup>42</sup>, is a good case in point. In 1971, its linkage to Pep C was recognized<sup>43</sup>, and when in 1972 Pep C was assigned to chromosome 1 by somatic cell genetics, the Rh gene was also assigned<sup>44</sup>. In many instances, the chromosome assignment of a mutant or polymorphic trait segregating in families has been facilitated by a linkage association with a second gene which had been assigned previously by somatic cell genetics. This is a promising development, as more than 1,000 unassigned human genes have been described and catalogued, many of which produce genetic disease<sup>44</sup>.

One can expect the assignment of complex organismal traits to chromosomes to be accelerated through the mapping of restriction fragments by somatic cell genetics. Restriction fragment mapping is accomplished by Southern blotting analysis, hybridizing labelled fragments to DNA preparations derived from interspecific somatic cell hybrids carrying one or a few donor chromosomes, assuming there are differences between the lengths of the homologous fragments of the donor and recipient species. Subsequently, linkage tests or in situ hybridization mapping would provide a regional assignment within a particular chromosome for a specific sequence. If several hundred restriction fragment markers selected randomly were mapped, they would be spaced at approximately 5-10 centimorgan intervals. It can be argued on theoretical grounds that such restriction fragment markers will be highly polymorphic within the human species 46. Wyman and White 47 have reported one restriction fragment length polymorphism in man which fits this description. DeMartinville et al. 58 have recently mapped this marker to human chromosome 14. Co-segregation within a family of a complex trait (a trait expressed only at the organismal level) and a specific restriction fragment polymorphism, previously assigned to a specific chromosome, would allow that trait to be mapped.

In practice, this approach to human gene mapping could be carried out in the following way. DNA samples can be collected from members of families segregating genes which control the expression of complex traits or disease conditions, analysed for restriction fragment polymorphisms, and the resulting polymorphisms tested for linkage with other co-segregating traits. In this way, a variety of genes amenable only to mendelian analysis can be assigned positions on the human gene map. Using this approach, it should by feasible eventually to map the chromosomal locations of all the major genetic disease genesfor example, Huntington's chorea, cystic fibrosis and xeroderma pigmentosum. One of the practical advantages of the restriction site polymorphism mapping technique is that a single methodological procedure can be used to study many genetic polymorphisms within a single kindred. In this manner, the restriction fragment length polymorphism method is similar to the standard practice of using serological tests for studying linkage relationships in human families.

Several techniques are available for the isolation of cloned DNA probes whose corresponding homologous sequences can be mapped. Some of these probes will represent sequences within genes of known function (for example, the globins or the insulins), while others will be of unknown function when first isolated. Fragments of both known and unknown function can be mapped and used as genetic markers equally well. To serve as discrete markers, the cloned fragments (probes) should be unique, single copy sequences. These can be obtained conveniently in cDNA libraries<sup>49</sup>. The cDNA probes have the additional advantage that they represent structural gene products and their biological function will ultimately become known. The cDNA probes will also be characterized to a degree by their tissues of origin. It is also possible to isolate DNA probes from genomic libraries, but in these instances the probability of recovering redundant sequences is significantly increased. Genomic sequences may, however, be subcloned,

subfragments tested for redundancy, and the desired unique sequences isolated.

Once a gene controlling a complex trait or genetic disease is mapped to a chromosomal region, additional restriction fragment markers can be mapped into that region using somatic cell genetics techniques. For example, Gusella et al.50 have produced cloned gene libraries from a man × mouse hybrid cell population carrying human chromosome 11 as a single donor chromosome. Such a library would contain fragments predominantly from the total mouse genome and a smaller set of clones containing DNA fragments from human chromosome 11. A subset of the human fragment clones will also contain repetitive human DNA species which can be detected by colony hybridization with labelled total human DNA. Gusella showed that in many instances the human repetitive sequences were contiguous with unique sequences. The single copy sequences could be isolated and then used as unique probes for the detection of their corresponding homologous sequences within chromosome 11. These were shown by direct analysis to map to chromosome 11 as expected. Subsequent regional mapping experiments assigned four such fragments to different regions of the chromosome. Thus, it is possible, using a cell hybrid which retains a defined donor genomic segment, to isolate cloned gene fragments which map specifically to that segment.

### Application of gene mapping to prenatal diagnosis

The ability to map restriction fragment markers randomly throughout the genome and to specific regions of chromosomes has obvious important implications for prenatal diagnosis, genetic screening of populations and paternity testing. Restriction fragment length polymorphisms closely linked to mutant alleles have already been used to predict genetic disease prenatally. In the case of sickle cell anaemia, Kan and Dozy have reported a polymorphism at a HpaI site 5,000 bp distant from the 3' end of the  $\alpha$ -globin gene<sup>51</sup>. When this site is present, a 7-kb fragment detectable with  $\alpha$ -globin probes is generated; when absent, a 13-kb fragment is produced. The HpaI site, which is responsible for the restriction fragment length polymorphism, is tightly linked to the  $\alpha$ -globin locus (5,000 bp is equivalent to 0.005 centimorgans) so that the probability of recombination between the HpaI site and the  $\alpha$ -globin locus is essentially zero. Therefore, if the polymorphism is present in heterozygous conditions in a family at risk for sickle cell disease, and if appropriate information exists to establish the linkage relationships between the polymorphic site and the  $\alpha$ -globin alleles in the parents, the restriction fragment polymorphism can be used to predict the  $\alpha$ -globin genotype of the fetus.

Kan and Dozy have calculated that 60% of black families in North America at risk for sickle cell anaemia are amenable to this kind of predictive analysis. Other polymorphisms in or around the  $\alpha$ -globin locus can be used for the same purpose, and serve to increase the number of families which might benefit from the procedure<sup>52</sup>. Geever et al.<sup>53</sup> have recently reported a restriction site change which is directly related to the nucleotide base substitution responsible for the conversion of the wild-type  $\alpha$ -globin gene to the sickle cell anaemia mutated version. In this case, there is a one to one relationship between the restriction pattern and mutant gene, thereby obviating the necessity of testing for linked polymorphisms in family members. Such direct tests for the presence or absence of gene mutations are likely to be the preferred prenatal diagnostic tests of the future.

The  $\alpha$ -globin example must not be taken as a representative case. The biochemical function of the  $\alpha$ -globin complex is known and more than 100 kb of the  $\alpha$ -globin region has been cloned. On the other hand, the biochemical lesions of many disease genes are unknown, and strategies for cloning these genes have not yet been devised. It should be possible to establish linkage between such a disease gene and a restriction fragment marker mendelian mapping, but finding tight linkage relationships of 1 centimorgan or less will be time consuming

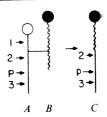


Fig. 3 Breakage-translocation method of establishing gene linkage relationships. A, donor chromosome; B, recipient chromosome; C, translocated chromosome resulting from exchange between the donor and recipient chromosomes. 1-3, Constitutive genes; P, prototrophic gene. The reciprocal rearrangement product bearing the donor chromosome is presumed to be lost.

and costly. On the other hand, a linkage distance in excess of 1 centimorgan is unlikely to be acceptable for prenatal diagnosis, so that in many instances cloning of the disease gene may be a necessary step in developing a prenatal diagnostic test based on the detection of restriction fragment polymorphisms. Alternatively, predictive accuracy can be significantly improved by mapping restriction fragment markers to positions flanking the disease gene. Positive identification of a genetically diseased fetus would then depend on the parental configuration of the outside markers (the non-cross-over class). False negative predictions for the disease gene would in this case result from double cross-overs occurring in the intervals flanking it. However, the error frequency will be small and is a function of the size of the flanking genetic intervals. If the distance between the disease gene and each of the flanking markers was 1 centimorgan the probability of the double cross-over would be 0.0001. The mapping of multiple restriction fragment markers in the vicinity of a disease gene can be accomplished by means of somatic cell genetic procedures referred to above. The availability of numerous, closely linked, flanking markers would increase the probability of detecting the appropriately polymorphic combinations within any given family. Moreover, somatic cell genetic procedures can be used to determine the cis/trans relationship of flanking mankers within particular family members.

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### 40Ar/39Ar age spectra from the KBS Tuff, **Koobi Fora Formation**

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 $^{40}Ar/^{39}Ar$  age spectra on anorthoclase phenocrysts from three pumice clasts in the KBS Tuff yield nearly ideal flat patterns, providing good evidence that the samples have remained undisturbed since crystallization. The ages are concordant at  $1.88 \pm 0.02$  Myr, and confirm that the KBS Tuff, a key marker bed in the Koobi Fora Formation, northern Kenya, is now very well dated. These results resolve the conflict between earlier 40Ar/39Ar and conventional K-Ar dating measurements on the KBS Tuff.

THE KBS Tuff is an important marker bed in the Pliocene to Pleistocene sedimentary sequence deposited in a basin centred on lat.  $4^{\circ}\,N, long.\,36.3^{\circ}\,E,$  adjacent to the eastern shores of Lake Turkana, northern Kenya<sup>1-3</sup>. Vertebrate fossils, including hominids, have been found<sup>4-9</sup> in stratigraphic proximity to the KBS Tuff, at the base of which numerous stone tools have been recovered<sup>6</sup>. Thus it is of interest to establish accurately the age of the KBS Tuff, especially in regard to hominid evolution 10. There has been much controversy as to the age of this bed<sup>7-9,11-1</sup>. but recent dating studies on minerals separated from pumice clasts found within the KBS Tuff, have provided relatively consistent ages within the range  $1.85 \pm 0.1$  Myr<sup>13-15</sup>. The most precise age estimate of 1.89 ± 0.01 Myr was based on conventional K-Ar isotopic age measurements on anorthoclase feldspar<sup>13</sup>.

An unresolved problem concerns understanding the meaning of the ages for the KBS Tuff reported by Fitch, Miller and coworkers 11,16-19, who mainly used the 40Ar/39Ar dating technique on anorthoclase separated from pumice clasts. The  $^{40}$ Ar/ $^{39}$ Ar total fusion ages ranged from  $0.52 \pm 0.33$  to  $2.6 \pm 0.3$ Myr, and the step heating measurements yielded complex age spectra, interpreted 11,17-19 as indicating marked disturbance of the anorthoclase subsequent to its crystallization. They suggested that crystallization occurred ~2.48 Myr ago, with deposition in the KBS Tuff shortly thereafter, followed by thermal overprinting at various times, especially at ~1.8 and

Here I present results of dating of anorthoclase separated from three pumice clasts found within the KBS Tuff, using the  $^{40}\mbox{Ar}/^{39}\mbox{Ar}$  total fusion and age spectrum techniques. These data provide strong evidence that the samples have remained undisturbed since crystallization, which occurred 1.88 ± 0.02 Myr ago.

### **KBS Tuff**

The KBS Tuff occurs within an essentially flatlying sequence of sediments, little more than 300-m thick, that crops out adjacent to Lake Turkana over an area at least 80 km (north-south) by 30 km (east–west). The KBS Tuff, commonly  $\sim 1$ -m thick, is the topmost unit of the lower member of the Koobi Fora Formation, and is overlain by no more than 125 m of sediment<sup>1-3</sup>. In common with other tuffs in the sequence, the KBS Tuff was transported by and deposited from water3, and consists mainly of rhyolitic glass with some admixture of early Palaeozoic and Tertiary detrital material.

Locally within the KBS Tuff, pumice clasts are found, regarded as products of the same volcanic eruptions that produced the bulk of the tuff<sup>3</sup>. Pumice clasts are used for the isotopic dating because they are less likely to be contaminated by old detrital material compared with the enclosing tuff. Here I have used anorthoclase separated from three pumice clasts, previously dated by the conventional K-Ar method<sup>13</sup>. Sample 78-1038 is from the KBS Tuff in area 105 (grid ref. HBH $\sim$ 730497), adjacent to its type locality, whereas samples 78-1047 and 79-14 are from a correlative of the KBS Tuff in area 131 (grid ref. HBH ~ 774594), about 10 km north-northeast of the locality for sample 78-1038. Geochemical data<sup>20</sup> from pumice clasts confirm that it is the same tuff in areas 105 and 131, but that the tuff in area 112 which yielded 13 similar K-Ar ages is not likely to be the same bed as the type KBS Tuff.

#### Methods

In the  $^{40}\text{Ar}/^{39}\text{Ar}$  dating method,  $^{39}\text{Ar}$  is generated from  $^{39}\text{K}$  in the sample by fast neutrons in a nuclear reactor, following which Ar is extracted in a high vacuum system and analysed isotopically, allowing simultaneous determination of K and

Table 1 40 Ar/39 Ar isotopic data and ages on anorthoclase from pumice clasts, KBS Tuff, Koobi Fora Formation, Kenya

mperature (°C)	<sup>39</sup> Ar (10 <sup>-15</sup> mol)	<sup>37</sup> Ar (10 <sup>-16</sup> mol)	<sup>36</sup> Ar (10 <sup>-17</sup> mol)	<sup>40</sup> Ar (10 <sup>-15</sup> mol)	(100 Rad <sup>40</sup> Ar)/ (Total <sup>40</sup> Ar)	Calculated (Myr)
	orthociase, step heat 1, 216	)350 um. 1.240 g. J = (	0.000646			
600	46±1	$127 \pm 80$	$329 \pm 10$	$1,063 \pm 1$	8.7	$2.315 \pm 0.7$
700	$230 \pm 1$	128± 53	$185 \pm 5$	$951 \pm 1$	42.3	$2.037 \pm 0.0$
770 770	474±1		156± 6	$1,271 \pm 1$	62.9	$1.967 \pm 0.0$
	$627 \pm 1$	222± 50	134± 6	$1,426 \pm 1$	71.4	$1.892 \pm 0.0$
820		2222 30	130± 3	1,686 ± 1	76.3	$1.884 \pm 0.0$
870	795±1			$1,652 \pm 2$	76.1	$1.830 \pm 0.0$
915	801 ± 1	128 ± 128	129 ± 6		78.5	1.836±0.0
940	$984 \pm 1$	$306 \pm 76$	139± 4	1,977±1	79.6	$1.867 \pm 0.0$
970	859 ± 1	$104 \pm 92$	$114 \pm 1$	$1,728 \pm 1$		
1,000	$1,165 \pm 2$	49± 86	$128 \pm 6$	$2,274 \pm 1$	82.4	$1.873 \pm 0.0$
1,040	$1,326 \pm 1$	$187 \pm 93$	126± 3	$2,533 \pm 2$	84.3	$1.877 \pm 0.0$
1,070	$1,236 \pm 2$	259± 66	$124 \pm 6$	$2,378 \pm 4$	83.7	$1.876 \pm 0.0$
1,110	$1,197 \pm 1$	$121 \pm 280$	107 ± 4	$2,308 \pm 1$	85.3	$1.915 \pm 0.0$
	(~910)	gas lost		ŕ		
1,160		gas rost	147± 5	$2,720 \pm 4$	83.1	$1.894 \pm 0.0$
1,225	1,389 ± 2	120 : 100	521 ± 9	$2,850 \pm 2$	45.5	$1.931 \pm 0.0$
1,360	782±1	$130 \pm 108$			25.3	2.153±0.
1,470	$74 \pm 1$	402 ± 36	$137 \pm 3$	540 ± 1	23.3	
Total	11,985	2,163	2,606	27,357		1.89 ±0.0
.1038 (KF38) An	orthoclase, step heat 2, 21	0-350 µm, 0.648 g, J =	0.000644			
760	118±1	193 ± 70	$295 \pm 2$	$1,088 \pm 1$	20.0	2.149 ± 0.0
830	$302 \pm 1$	$113 \pm 18$	$144 \pm 4$	$911 \pm 1$	52.9	$1.856 \pm 0.0$
890	$408 \pm 1$	$64 \pm 145$	93 ± 6	938±1	69.8	$1.864 \pm 0.0$
930	442±1	$64 \pm 134$	90± 4	966 ± 1	71.7	$1.816 \pm 0.0$
970	580±1		87± 3	$1,212 \pm 1$	77.9	$1.892 \pm 0.0$
		152 ± 129	93± 4	$1,165 \pm 1$	75.8	$1.868 \pm 0.6$
1,000	548±1		76± 5	$1,103\pm 1$ $1,203\pm 1$	80.4	1.906±0.
1,030	$590 \pm 1$	142± 66			82.1	1.887±0.0
1,065	$610 \pm 1$	$131 \pm 49$	$70 \pm 4$	$1,208 \pm 1$		
1,100	$751 \pm 1$	$73 \pm 111$	$160 \pm 2$	$1,686 \pm 1$	71.2	$1.855 \pm 0.0$
1,150	849±1	$57 \pm 139$	$122 \pm 5$	$1,749 \pm 1$	78.5	$1.878 \pm 0.9$
1,200	$1,188 \pm 1$	$201 \pm 98$	$142 \pm 4$	$2,369 \pm 1$	81.5	$1.885 \pm 0.0$
1,430	$468 \pm 1$	$326 \pm 178$	$295 \pm 3$	$1,640 \pm 3$	46.4	$1.887 \pm 0.0$
Total	6,854	1,516	1,667	16,135		$1.88 \pm 0.6$
i-1038 (KF38) An 1,500	northoclase, total fusion, 0. 1,413 ± 1	143 g, $J = 0.000646$ 130 ± 49	257± 4	$3,089 \pm 1$	74.6	1.900±0.
•						
	northoclase, step heat, 210- $34 \pm 1$	$-350 \mu m$ , 1.367 g, $J = 0$ . $8 \pm 8$	.000658 584 ± 6	1,779 ± 2	3.0	$1.846 \pm 0$
600		61 6	173 ± 2	547±1	6.5	$1.321 \pm 0$
720	$32 \pm 1$	***				1.977±0.
790	$500 \pm 1$	$56 \pm 20$	618± 6	$2,666 \pm 3$	31.2	
850	$771 \pm 1$	$112 \pm 11$	$594 \pm 6$	$2,995 \pm 3$	41.0	$1.890 \pm 0$
900	991 ± 1	82 ± 11	553 ± 8	$3,218 \pm 3$	48.6	$1.874 \pm 0.$
940	4 4 4 4 4 4	107 : 24	$419 \pm 7$			$1.881 \pm 0.$
	$1.179 \pm 1$	$107 \pm 24$	71/+ /	$3,125 \pm 3$	59.8	T.00 T in 0.
970	$1,179 \pm 1$ $1,225 \pm 1$	107± 24 138± 11		$3,125 \pm 3$ $3,093 \pm 3$	59.8 63.6	
970	$1,225 \pm 1$	138 ± 11	$374 \pm 3$	$3,093 \pm 3$	63.6	$1.907 \pm 0$
1,000	$1,225 \pm 1$ $1,280 \pm 1$	$138 \pm 11$ $135 \pm 29$	$374 \pm 3$ $399 \pm 8$	$3,093 \pm 3$ $3,208 \pm 3$	63.6 62.6	$1.907 \pm 0.$ $1.861 \pm 0.$
1,000 1,045	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$	138± 11 135± 29 127± 25	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$	3,093 ± 3 3,208 ± 3 3,580 ± 4	63.6 62.6 67.1	$1.907 \pm 0.$ $1.861 \pm 0.$ $1.866 \pm 0.$
1,000 1,045 1,070	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$	$ \begin{array}{r} 138 \pm 11 \\ 135 \pm 29 \\ 127 \pm 25 \\ 115 \pm 21 \end{array} $	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$	$3,093 \pm 3$ $3,208 \pm 3$ $3,580 \pm 4$ $3,602 \pm 4$	63.6 62.6 67.1 68.8	1.907±0. 1.861±0. 1.866±0. 1.897±0.
1,000 1,045 1,070 1,130	$ 1,225 \pm 1  1,280 \pm 1  1,527 \pm 2  1,551 \pm 2  1,799 \pm 2 $	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$ $425 \pm 6$	$3,093 \pm 3$ $3,208 \pm 3$ $3,580 \pm 4$ $3,602 \pm 4$ $4,095 \pm 4$	63.6 62.6 67.1 68.8 68.5	1.907 ± 0. 1.861 ± 0. 1.866 ± 0. 1.897 ± 0. 1.852 ± 0.
1,000 1,045 1,070 1,130	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$	$ \begin{array}{r} 138 \pm 11 \\ 135 \pm 29 \\ 127 \pm 25 \\ 115 \pm 21 \end{array} $	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$	$3,093 \pm 3$ $3,208 \pm 3$ $3,580 \pm 4$ $3,602 \pm 4$	63.6 62.6 67.1 68.8	1.907 ± 0. 1.861 ± 0. 1.866 ± 0. 1.897 ± 0. 1.852 ± 0.
1,000 1,045 1,070 1,130 1,180	$ 1,225 \pm 1  1,280 \pm 1  1,527 \pm 2  1,551 \pm 2  1,799 \pm 2 $	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$ $425 \pm 6$	$3,093 \pm 3$ $3,208 \pm 3$ $3,580 \pm 4$ $3,602 \pm 4$ $4,095 \pm 4$	63.6 62.6 67.1 68.8 68.5 68.0	$ 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.852 \pm 0. \\ 1.872 \pm 0. $
1,000 1,045 1,070 1,130 1,180 1,210	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$ $144 \pm 3$	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$ $425 \pm 6$	$3,093 \pm 3$ $3,208 \pm 3$ $3,580 \pm 4$ $3,602 \pm 4$ $4,095 \pm 4$	63.6 62.6 67.1 68.8 68.5	$ 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.852 \pm 0. \\ 1.872 \pm 0. $
1,000 1,045 1,070 1,130 1,180 1,210 1,250	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$	$   \begin{array}{r}     138 \pm 11 \\     135 \pm 29 \\     127 \pm 25 \\     115 \pm 21 \\     153 \pm 23 \\     144 \pm 3 \\     gas lost \\     26 \pm 20   \end{array} $	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5	3,093 ± 3 3,208 ± 3 3,580 ± 4 3,602 ± 4 4,095 ± 4 4,292 ± 4	63.6 62.6 67.1 68.8 68.5 68.0	1.907 ± 0. 1.861 ± 0. 1.866 ± 0. 1.897 ± 0. 1.852 ± 0. 1.872 ± 0.
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$ $144 \pm 3$ gas lost	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$ $425 \pm 6$ $453 \pm 7$	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4 2,319±2	63.6 62.6 67.1 68.8 68.5 68.0	$   \begin{array}{c}     1.907 \pm 0. \\     1.861 \pm 0. \\     1.866 \pm 0. \\     1.897 \pm 0. \\     1.852 \pm 0. \\     1.872 \pm 0. \\   \end{array} $ $   \begin{array}{c}     1.938 \pm 0. \\     1.656 \pm 0. \\   \end{array} $
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$ $144 \pm 3$ $gas lost$ $26 \pm 20$ $10 \pm 19$ $1,213$	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$ $425 \pm 6$ $453 \pm 7$ $480 \pm 5$ $1,052 \pm 8$	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4 2,319±2 3,297±5	63.6 62.6 67.1 68.8 68.5 68.0	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.852 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$ $144 \pm 3$ $gas lost$ $26 \pm 20$ $10 \pm 19$ $1,213$	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$ $425 \pm 6$ $453 \pm 7$ $480 \pm 5$ $1,052 \pm 8$	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4 2,319±2 3,297±5	63.6 62.6 67.1 68.8 68.5 68.0	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \end{array}$
1,000 1,045 1,070 1,130 1,130 1,210 1,250 1,320 Total 6-1047 (KF47) An	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ <b>northoclase, total fusion, 0.</b> $6,515 \pm 6$	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$ $144 \pm 3$ $gas lost$ $26 \pm 20$ $10 \pm 19$ $1,213$ $625 g, J = 0.000661$ $541 \pm 26$	$374 \pm 3$ $399 \pm 8$ $390 \pm 5$ $371 \pm 3$ $425 \pm 6$ $453 \pm 7$ $480 \pm 5$ $1,052 \pm 8$ $6,885$	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4 2,319±2 3,297±5 41,816	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6	1.907±0. 1.861±0. 1.865±0. 1.897±0. 1.872±0. 1.938±0. 1.656±0. 1.88 ±0.
1,000 1,045 1,070 1,130 1,130 1,210 1,250 1,320 Total 6-1047 (KF47) An	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ aorthoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 196 \times 1	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41.816$ $15.220 \pm 7$ $1.934 \pm 3$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6	$   \begin{array}{c}     1.907 \pm 0. \\     1.861 \pm 0. \\     1.866 \pm 0. \\     1.897 \pm 0. \\     1.872 \pm 0. \\     1.872 \pm 0. \\     1.878 \pm 0. \\     1.881 \pm 0. \\     2.474 \pm 0. \\   \end{array} $
1,000 1,045 1,070 1,130 1,130 1,210 1,250 1,320 Total 6-1047 (KF47) An 1,500 0-14 (7722-1071)	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ northoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 10	$138 \pm 11$ $135 \pm 29$ $127 \pm 25$ $115 \pm 21$ $153 \pm 23$ $144 \pm 3$ $gas lost$ $26 \pm 20$ $10 \pm 19$ $1,213$ $625 g, J = 0.000661$ $541 \pm 26$	374 ± 3 399 ± 8 390 ± 5 371 ± 3 425 ± 6 453 ± 7 480 ± 5 1,052 ± 8 6,885 1,525 ± 8	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41.816$ $15,220 \pm 7$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total -1047 (KF47) An 1,500 -14 (7722-1071) 600 700	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ aorthoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 196 \times 1	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41.816$ $15.220 \pm 7$ $1.934 \pm 3$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.355 \pm 0. \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total -1047 (KF47) An 1,500 -14 (7722-1071) . 600 700 730	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ northoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 10. $96 \pm 1$ $80 \pm 1$ $116 \pm 1$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26	374 ± 3 399 ± 8 390 ± 5 371 ± 3 425 ± 6 453 ± 7 480 ± 5 1,052 ± 8 6,885 1,525 ± 8 =0.000568 575 ± 4 270 ± 8 217 ± 10	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41.816$ $15.220 \pm 7$ $1.934 \pm 3$ $982 \pm 1$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.865 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total -1047 (KF47) An 1,500 -14 (7722-1071) 600 730 760	$\begin{array}{c} 1,225\pm 1 \\ 1,280\pm 1 \\ 1,527\pm 2 \\ 1,551\pm 2 \\ 1,799\pm 2 \\ 1,851\pm 2 \\ (\sim 840) \\ 545\pm 1 \\ 133\pm 1 \\ 13,418 \\ \\ \textbf{northoclase, total fusion, 0.} \\ 6,515\pm 6 \\ \textbf{Anorthoclase, step heat, 10.} \\ 96\pm 1 \\ 80\pm 1 \\ 116\pm 1 \\ 274\pm 1 \\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J =	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4  2,319±2 3,297±5 41,816  15,220±7  1,934±3 982±1 898±1 1,369±1	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 2.252 \pm 0. \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total -1047 (KF47) An 1,500 -14 (7722-1071) 600 730 760 820	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ aorthoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 1 $96 \pm 1$ $80 \pm 1$ $116 \pm 1$ $274 \pm 1$ $1,259 \pm 1$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41.816$ $15.220 \pm 7$ $1.934 \pm 3$ $982 \pm 1$ $898 \pm 1$ $1.369 \pm 1$ $4.005 \pm 3$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.241 \pm 0. \\ 2.241 \pm 0. \\ 1.941 \pm 0. \\$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total -1047 (KF47) An 1,500 -14 (7722-1071) 600 700 730 760 820 880	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ aorthoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 1 $96 \pm 1$ $80 \pm 1$ $116 \pm 1$ $274 \pm 1$ $1,259 \pm 1$ $1,258 \pm 2$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J =	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41.816$ $15.220 \pm 7$ $1.934 \pm 3$ $982 \pm 1$ $898 \pm 1$ $1.369 \pm 1$ $4.005 \pm 3$ $3.463 \pm 5$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.656 \pm 0. \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 2.252 \pm 0. \\ 1.941 \pm 0. \\ 1.886 \pm 0. \end{array}$
1,000 1,045 1,070 1,130 1,130 1,210 1,250 1,320 Total  -1047 (KF47) An 1,500  -14 (7722-1071) -600 -730 -760 820 880 930	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ aorthoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 10. $96 \pm 1$ $80 \pm 1$ $116 \pm 1$ $274 \pm 1$ $1,259 \pm 1$ $1,258 \pm 2$ $2,259 \pm 2$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J =	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 = 0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4  2,319±2 3,297±5 41,816  15,220±7  1,934±3 982±1 898±1 1,369±1 4,005±3 3,463±5 5,557±5	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 2.252 \pm 0. \\ 1.941 \pm 0. \\ 1.886 \pm 0. \\ 1.868 \pm 0. \\ \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  3-1047 (KF47) An 1,500  3-14 (7722-1071) 600 730 760 820 880 930 965	$\begin{array}{c} 1,225\pm 1\\ 1,280\pm 1\\ 1,527\pm 2\\ 1,551\pm 2\\ 1,799\pm 2\\ 1,851\pm 2\\ (\sim 840)\\ 545\pm 1\\ 133\pm 1\\ 13,418\\ \\ \textbf{northoclase, total fusion, 0.}\\ 6,515\pm 6\\ \textbf{Anorthoclase, step heat, 16}\\ 96\pm 1\\ 80\pm 1\\ 116\pm 1\\ 274\pm 1\\ 1,259\pm 1\\ 1,258\pm 2\\ 2,259\pm 2\\ 1,593\pm 1\\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 —	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ 41.816 $15,220 \pm 7$ $1,934 \pm 3$ $982 \pm 1$ $898 \pm 1$ $1.369 \pm 1$ $4.005 \pm 3$ $3.463 \pm 5$ $5.557 \pm 5$ $3.770 \pm 2$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.865 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 2.252 \pm 0. \\ 1.886 \pm 0. \\ 1.868 \pm 0. \\ \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  -1047 (KF47) An 1,500  -14 (7722-1071) 600 730 760 820 880 930	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ aorthoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 10. $96 \pm 1$ $80 \pm 1$ $116 \pm 1$ $274 \pm 1$ $1,259 \pm 1$ $1,258 \pm 2$ $2,259 \pm 2$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 — 51± 84	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9 209± 1	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4  2,319±2 3,297±5 41,816  15,220±7  1,934±3 982±1 898±1 1,369±1 4,005±3 3,463±5 5,557±5 3,770±2 2,765±3	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1 77.4 76.6	1.907±0. 1.861±0. 1.866±0. 1.879±0. 1.852±0. 1.872±0. 1.938±0. 1.656±0. 1.88 ±0. 2.474±0. 2.355±0. 2.241±0. 1.868±0. 1.868±0. 1.877±0. 1.858±0.
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  3-1047 (KF47) An 1,500  2-14 (7722-1071) 600 730 760 820 880 930 965 1,000	$\begin{array}{c} 1,225\pm 1\\ 1,280\pm 1\\ 1,527\pm 2\\ 1,551\pm 2\\ 1,799\pm 2\\ 1,851\pm 2\\ (\sim 840)\\ 545\pm 1\\ 133\pm 1\\ 13,418\\ \\ \textbf{northoclase, total fusion, 0.}\\ 6,515\pm 6\\ \textbf{Anorthoclase, step heat, 16}\\ 96\pm 1\\ 80\pm 1\\ 116\pm 1\\ 274\pm 1\\ 1,259\pm 1\\ 1,258\pm 2\\ 2,259\pm 2\\ 1,593\pm 1\\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 —	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ 41.816 $15,220 \pm 7$ $1,934 \pm 3$ $982 \pm 1$ $898 \pm 1$ $1.369 \pm 1$ $4.005 \pm 3$ $3.463 \pm 5$ $5.557 \pm 5$ $3.770 \pm 2$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.252 \pm 0. \\ 1.941 \pm 0. \\ 1.886 \pm 0. \\ 1.877 \pm 0. \\ 1.877 \pm 0. \\ 1.858 \pm 0. \\ \end{array}$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  -1047 (KF47) An 1,500  -14 (7722-1071) 600 700 730 760 820 880 930 9365 1,000 1,045	$\begin{array}{c} 1,225\pm 1\\ 1,280\pm 1\\ 1,527\pm 2\\ 1,551\pm 2\\ 1,799\pm 2\\ 1,851\pm 2\\ (\sim 840)\\ 545\pm 1\\ 133\pm 1\\ 13,418\\ \\ \textbf{northoclase, total fusion, 0.}\\ 6,515\pm 6\\ \\ \textbf{Anorthoclase, step heat, 1}\\ 96\pm 1\\ 80\pm 1\\ 116\pm 1\\ 274\pm 1\\ 1,259\pm 1\\ 1,258\pm 2\\ 2,259\pm 2\\ 1,593\pm 1\\ 1,168\pm 1\\ 1,139\pm 1\\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 — 51± 84	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9 209± 1 197± 3	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4  2,319±2 3,297±5 41,816  15,220±7  1,934±3 982±1 898±1 1,369±1 4,005±3 3,463±5 5,557±5 3,770±2 2,765±3 2,704±2	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1 77.4	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 1.886 \pm 0. \\ 1.868 \pm 0. \\ 1.875 \pm 0. \\ 1.878 \pm 0. \\ 1.888 \pm 0. \\$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  -1047 (KF47) An 1,500  -14 (7722-1071) 600 700 730 760 820 880 930 965 1,000 1,045 1,100	$1,225 \pm 1$ $1,280 \pm 1$ $1,527 \pm 2$ $1,551 \pm 2$ $1,799 \pm 2$ $1,851 \pm 2$ $(\sim 840)$ $545 \pm 1$ $133 \pm 1$ $13,418$ aorthoclase, total fusion, 0. $6,515 \pm 6$ Anorthoclase, step heat, 10. $96 \pm 1$ $80 \pm 1$ $116 \pm 1$ $274 \pm 1$ $1,259 \pm 1$ $1,258 \pm 2$ $2,259 \pm 2$ $1,593 \pm 1$ $1,168 \pm 1$ $1,139 \pm 1$ $494 \pm 1$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 — 51± 84 141±132 —	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9 209± 1 197± 3 106± 6	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41,816$ $15,220 \pm 7$ $1,934 \pm 3$ $982 \pm 1$ $898 \pm 1$ $1,369 \pm 1$ $4.005 \pm 3$ $3.463 \pm 5$ $5.557 \pm 5$ $3.770 \pm 2$ $2.765 \pm 3$ $2.704 \pm 2$ $1,250 \pm 1$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1 77.4 76.6 77.4 73.8	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 1.868 \pm 0. \\ 1.872 \pm 0. \\ 1.868 \pm 0. \\ 1.872 \pm 0. \\ 1.858 \pm 0. \\ 1.872 \pm 0. \\ 1.858 \pm 0. \\ 1.872 \pm 0. \\ 1.858 \pm 0. \\ 1.872 \pm 0. \\ 1.883 \pm 0. \\ 1.912 \pm 0. \\$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  3-1047 (KF47) An 1,500  2-14 (7722-1071) 600 730 760 820 880 930 965 1,000 1,045 1,100 1,150	$\begin{array}{c} 1,225\pm 1\\ 1,280\pm 1\\ 1,527\pm 2\\ 1,551\pm 2\\ 1,799\pm 2\\ 1,851\pm 2\\ (\sim 840)\\ 545\pm 1\\ 133\pm 1\\ 13,418\\ \\ \textbf{northoclase, total fusion, 0.}\\ 6,515\pm 6\\ \textbf{Anorthoclase, step heat, 16}\\ 96\pm 1\\ 80\pm 1\\ 116\pm 1\\ 274\pm 1\\ 1,259\pm 1\\ 1,258\pm 2\\ 2,259\pm 2\\ 1,593\pm 1\\ 1,168\pm 1\\ 1,139\pm 1\\ 494\pm 1\\ 946\pm 1\\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 — 51± 84 141±132 — 206±127	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9 209± 1 197± 3 106± 6 228± 9	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41.816$ $15.220 \pm 7$ $1.934 \pm 3$ $982 \pm 1$ $898 \pm 1$ $1.369 \pm 1$ $4.005 \pm 3$ $3.463 \pm 5$ $5.557 \pm 5$ $3.770 \pm 2$ $2.765 \pm 3$ $2.704 \pm 2$ $1.250 \pm 1$ $2.493 \pm 1$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1 77.4 76.6 77.4 73.8 72.0	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.656 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 2.252 \pm 0. \\ 1.841 \pm 0. \\ 1.868 \pm 0. \\ 1.877 \pm 0. \\ 1.858 \pm 0. \\ 1.877 \pm 0. \\ 1.858 \pm 0. \\ 1.872 \pm 0. \\ 1.843 \pm 0. \\ 1.844 \pm 0. \\$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  3-1047 (KF47) An 1,500  3-14 (7722-1071) 600 700 730 760 820 880 930 965 1,000 1,045 1,100 1,150 1,250	$\begin{array}{c} 1,225\pm 1\\ 1,280\pm 1\\ 1,527\pm 2\\ 1,551\pm 2\\ 1,799\pm 2\\ 1,851\pm 2\\ (\sim 840)\\ 545\pm 1\\ 133\pm 1\\ 13,418\\ \\ \textbf{northoclase, total fusion, 0.}\\ 6,515\pm 6\\ \\ \textbf{Anorthoclase, step heat, 1}\\ 96\pm 1\\ 80\pm 1\\ 116\pm 1\\ 274\pm 1\\ 1,259\pm 1\\ 1,259\pm 1\\ 1,259\pm 2\\ 2,259\pm 2\\ 1,593\pm 1\\ 1,168\pm 1\\ 1,139\pm 1\\ 494\pm 1\\ 946\pm 1\\ 997\pm 1\\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 — 51± 84 141±132 — 206±127 187±142	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9 209± 1 197± 3 106± 6 228± 9 557±10	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4  2,319±2 3,297±5 41,816  15,220±7  1,934±3 982±1 898±1 1,369±1 4,005±3 3,463±5 5,557±5 3,770±2 2,765±3 2,704±2 1,250±1 2,493±1 3,736±2	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1 77.4 76.6 77.4 73.8 72.0 55.2	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.252 \pm 0. \\ 1.941 \pm 0. \\ 1.868 \pm 0. \\ 1.868 \pm 0. \\ 1.877 \pm 0. \\ 1.858 \pm 0. \\ 1.812 \pm 0. \\ 1.822 \pm 0. \\ 1.842 \pm 0. \\$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  -1047 (KF47) An 1,500  -14 (7722-1071) 600 700 730 760 820 880 930 965 1,000 1,045 1,100 1,150 1,250 1,400	$\begin{array}{c} 1,225\pm 1\\ 1,280\pm 1\\ 1,527\pm 2\\ 1,551\pm 2\\ 1,799\pm 2\\ 1,851\pm 2\\ (\sim 840)\\ 545\pm 1\\ 133\pm 1\\ 13,418\\ \\ \hline \text{northoclase, total fusion, 0.}\\ 6,515\pm 6\\ \hline \textbf{Anorthoclase, step heat, 1}\\ 96\pm 1\\ 80\pm 1\\ 116\pm 1\\ 274\pm 1\\ 1,259\pm 1\\ 1,258\pm 2\\ 2,259\pm 2\\ 1,593\pm 1\\ 1,168\pm 1\\ 1,139\pm 1\\ 494\pm 1\\ 946\pm 1\\ 997\pm 1\\ 78\pm 1\\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 — 51± 84 141±132 — 206±127 187±142 90±110	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9 209± 1 197± 3 106± 6 228± 9 557±10 1,090± 7	$3.093 \pm 3$ $3.208 \pm 3$ $3.580 \pm 4$ $3.602 \pm 4$ $4.095 \pm 4$ $4.095 \pm 4$ $4.292 \pm 4$ $2.319 \pm 2$ $3.297 \pm 5$ $41,816$ $15,220 \pm 7$ $1,934 \pm 3$ $982 \pm 1$ $898 \pm 1$ $1,369 \pm 1$ $4.005 \pm 3$ $3.463 \pm 5$ $5,557 \pm 5$ $3.770 \pm 2$ $2,765 \pm 3$ $2,704 \pm 2$ $1,250 \pm 1$ $2,493 \pm 1$ $3,736 \pm 2$ $3,316 \pm 3$	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1 77.4 76.6 77.4 73.8 72.0	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.355 \pm 0. \\ 2.241 \pm 0. \\ 1.868 \pm 0. \\ 1.862 \pm 0. \\$
1,000 1,045 1,070 1,130 1,180 1,210 1,250 1,320 Total  -1047 (KF47) An 1,500  -14 (7722-1071) 600 700 730 760 820 880 930 965 1,000 1,045 1,100 1,150 1,250	$\begin{array}{c} 1,225\pm 1\\ 1,280\pm 1\\ 1,527\pm 2\\ 1,551\pm 2\\ 1,799\pm 2\\ 1,851\pm 2\\ (\sim 840)\\ 545\pm 1\\ 133\pm 1\\ 13,418\\ \\ \textbf{northoclase, total fusion, 0.}\\ 6,515\pm 6\\ \\ \textbf{Anorthoclase, step heat, 1}\\ 96\pm 1\\ 80\pm 1\\ 116\pm 1\\ 274\pm 1\\ 1,259\pm 1\\ 1,259\pm 1\\ 1,259\pm 2\\ 2,259\pm 2\\ 1,593\pm 1\\ 1,168\pm 1\\ 1,139\pm 1\\ 494\pm 1\\ 946\pm 1\\ 997\pm 1\\ \end{array}$	138± 11 135± 29 127± 25 115± 21 153± 23 144± 3 gas lost 26± 20 10± 19 1,213 625 g, J = 0.000661 541± 26 00-350 μm, 1.339 g, J = 251±175 — 352± 85 — 51± 84 141±132 — 206±127 187±142	374± 3 399± 8 390± 5 371± 3 425± 6 453± 7 480± 5 1,052± 8 6,885 1,525± 8 =0.000568 575± 4 270± 8 217±10 257± 9 538± 4 377± 8 466± 8 274± 9 209± 1 197± 3 106± 6 228± 9 557±10	3,093±3 3,208±3 3,580±4 3,602±4 4,095±4 4,292±4  2,319±2 3,297±5 41,816  15,220±7  1,934±3 982±1 898±1 1,369±1 4,005±3 3,463±5 5,557±5 3,770±2 2,765±3 2,704±2 1,250±1 2,493±1 3,736±2	63.6 62.6 67.1 68.8 68.5 68.0 38.4 5.6 67.6 12.0 18.8 28.3 44.0 59.5 66.9 74.1 77.4 76.6 77.4 73.8 72.0 55.2	$\begin{array}{c} 1.907 \pm 0. \\ 1.861 \pm 0. \\ 1.866 \pm 0. \\ 1.897 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.872 \pm 0. \\ 1.938 \pm 0. \\ 1.656 \pm 0. \\ 1.88 \pm 0. \\ 1.881 \pm 0. \\ 2.474 \pm 0. \\ 2.252 \pm 0. \\ 1.941 \pm 0. \\ 1.868 \pm 0. \\ 1.877 \pm 0. \\ 1.888 \pm 0. \\ 1.877 \pm 0. \\ 1.888 \pm 0. \\ 1.872 \pm 0. \\ 1.883 \pm 0. \\ 1.912 \pm 0. \\ 1.942 \pm 0. \\ 2.122 \pm 0. \\ 2.122 \pm 0. \end{array}$

 $<sup>\</sup>lambda = 5.543 \times 10^{-10} \, \mathrm{yr^{-1}}$ . Errors at level of 1 s.d. All data corrected for instrument discrimination. <sup>37</sup>Ar and <sup>39</sup>Ar corrected for decay. Line blanks have not been applied, but are as follows for <sup>40</sup>Ar: 78-1038 step heat 1, 2.5 × 10<sup>-13</sup> mol; 78-1038 step heat 2, 1.5 × 10<sup>-13</sup> mol; 78-1047 step heat,  $4 \times 10^{-13} \, \mathrm{mol}$ ; 79-14 step heat,  $3 \times 10^{-13} \, \mathrm{mol}$ . Reported absolute amounts have an additional untabulated uncertainty of  $\sim 10\%$ .

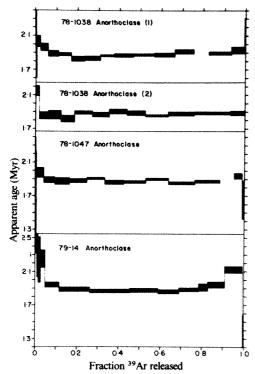


Fig. 1 40Ar/39Ar age spectra for anorthoclase from pumice clasts in the KBS Tuff. Data plotted as apparent age against fraction of 39Ar released. Uncertainty of the age for each step, at the level of 1 s.d., indicated by thickness of bar.

radiogenic Ar (<sup>40</sup>Ar\*) (ref. 21). The ratio <sup>40</sup>Ar\*/<sup>39</sup>Ar is proportional to age. The method is applied in two distinct ways, both of which are used here. First, the sample can be fused after irradiation and from the measured isotopic composition of the Ar an age can be derived, which normally agrees with the conventional K-Ar age. Second, after irradiation of the sample, the Ar can be released in stages by stepwise or incremental heating, commencing at a temperature well below that of fusion, and the gas evolved in successive steps analysed to provide a series of apparent ages. A plot of age versus fraction of <sup>39</sup>Ar released, an age spectrum, may be interpreted<sup>22</sup> in terms of distribution of <sup>40</sup>Ar\* and <sup>39</sup>Ar in the sample.

Anorthoclase should be nearly ideal for  $^{40}$ Ar/ $^{39}$ Ar age spectrum measurements as it remains stable in the vacuum system during much of the heating, so that diffusion is likely to be the major transport mechanism for extraction of Ar. The crystal fragments were kept relatively coarse, in the range  $100-350 \,\mu\text{m}$ , in the hope that if geologically-induced diffusion profiles for  $^{40}$ Ar\* are present, they would be revealed by the age spectrum measurements.

Irradiations for 30 h were carried out in a horizontal facility adjacent to, but not within the core of Hifar reactor<sup>23,24</sup>, where the fast neutron flux is  $\sim 4 \times 10^{12}$  neutrons cm<sup>-2</sup> s<sup>-1</sup>. The cylindrical reactor vessel of internal size ~31 mm (length) by 11 mm (diameter) was lined with Cd to reduce the (40Ar/39Ar) correction factor, measured by analysis of K2SO4 salt, to a low level<sup>25</sup>. Samples were placed in machined Al containers, with only one unknown in each reactor vessel in two separate containers of different size, sandwiched between those in which K<sub>2</sub>SO<sub>4</sub> was placed at either end of the vessel. Centrally located within each anorthoclase sample container was another cylindrical container in which the flux monitor, GA1550 biotite of K-Ar age 97.9 Myr (refs 23, 26), was placed. This geometrical arrangement of flux monitor and sample was developed<sup>27</sup> initially to overcome problems of large neutron flux gradients, but inversion of the reactor vessel at exactly halfway through the irradiation has virtually eliminated this difficulty, so that the neutron fluence can now be measured routinely to better than 1%

Samples for <sup>40</sup>Ar/<sup>39</sup>Ar age spectrum measurements were heated by means of a radiofrequency generator for 45-min

intervals at successively higher temperatures in the Ar extraction system. Temperatures were measured by thermocouple and optical pyrometer; increments between successive steps are probably correct to ~10 °C, but the actual temperatures given in Table 1 are only approximate, because of relatively large temperature gradients over the Mo crucible in which the sample was heated. The gas from each extraction was purified and the Ar transferred directly to a Micromass 12 mass spectrometer. operated in the static mode and at an accelerating potential of 2 kV. Peak hopping, by varying the magnetic field, is used to focus the ion beams on the Faraday cup. Data are taken digitally on-line with a Hewlett-Packard 1000 computer, in which all data reduction is carried out. The amount of gas extracted at each step averaged  $\sim 2 \times 10^{-12}$  mol <sup>40</sup>Ar, giving an ion beam of ~500 mV using a  $10^{11}$ - $\Omega$  resistor in the Cary  $4\overline{0}1$  electrometer. Background in the mass spectrometer was measured routinely but normally was negligible.

Peak height data from the mass spectrometer were extrapolated to the time of admission of the gas into the machine using a least squares procedure and corrections applied for machine discrimination, decay of  $^{37}\mathrm{Ar}$  and  $^{39}\mathrm{Ar}$  and for neutron-induced interfering isotopes. Correction factors applied were: ( $^{36}\mathrm{Ar}/^{37}\mathrm{Ar})_{\mathrm{Ca}} = 3.06 \times 10^{-4}$ ; ( $^{39}\mathrm{Ar}/^{37}\mathrm{Ar})_{\mathrm{Ca}} = 7.27 \times 10^{-4}$  (ref. 25), and ( $^{40}\mathrm{Ar}/^{39}\mathrm{Ar})_{\mathrm{K}} = 0.020 \pm 0.005$ , the average value measured on  $\mathrm{K}_2\mathrm{SO}_4$  during this work. For 78-1047 total fusion only, the value of the K correction factor was 0.066.

#### Results

Data are given in Table 1 as amounts of each Ar isotope, derived from the measured sensitivity of the mass spectrometer. The relative amounts quoted for a given analysis are precise to within the uncertainties, but the absolute amounts may only be accurate to  $\sim\!10\%$ . In a few cases almost all the  $^{37}\mathrm{Ar}$  had decayed before analysis, but as the K/Ca ratio of these feldspars is large (>50), corrections for neutron-induced Ar from Ca are small to negligible. Data in Table 1 are not corrected for line blank, because the blank accounts for more than half the  $^{36}\mathrm{Ar}$  in many analyses, and it is only known to  $\sim\!50\%$ . Information is given in Table 1 so that blank corrected data can be calculated if desired.

Ages listed in Table 1 are calculated on the assumption that the non-radiogenic or trapped Ar in the samples has the composition of atmospheric Ar in which  $^{40}$ Ar/ $^{36}$ Ar = 295.5. These calculated ages are also shown as age spectra in Fig. 1. ' In Fig. 2 data are shown for each step heating experiment on a correlation diagram with <sup>36</sup>Ar/<sup>40</sup>Ar as the ordinate and <sup>39</sup>Ar/<sup>40</sup>Ar as the abscissa <sup>28-30</sup>. If data from a step heating experiment define a good straight line on this diagram, it can be interpreted as a mixing line between non-radiogenic or trapped Ar, represented by a point on the ordinate, and Ar derived from K, represented by a point on the abscissa. Thus the isotopic composition of Ar for each gas fraction plots in Fig. 2 at a point determined by the proportion of the two end members. The intercept of the line with the ordinate defines the <sup>36</sup>Ar/<sup>40</sup>Ar ratio of the trapped component, and the intercept with the abscissa gives the <sup>39</sup>Ar/<sup>40</sup>Ar ratio, the reciprocal of which is proportional to age. This type of correlation diagram is preferred to the more common <sup>40</sup>Ar/<sup>36</sup>Ar versus <sup>39</sup>Ar/<sup>36</sup>Ar plot because problems associated with correlated errors are much less. Results of the regression analyses, using standard techniques<sup>31</sup>, are given in Table 2. In the regressions, the MSWD is a goodness of fit parameter; if this exceeds a value of  $\sim 2.5$  it can be concluded<sup>32</sup> that there is scatter of data points about the line significantly greater than can be accounted for by experimental error alone.

### Discussion

The  $^{40}$ Ar/ $^{39}$ Ar total fusion ages, measured on separate aliquots of the samples, agree well with one another, yielding a mean age of  $1.89 \pm 0.01$  Myr (Tables 1 and 2). In addition, the individual results are concordant with the K-Ar ages measured on the same samples (Table 2).

Table 2 Summary of 40 Ar/39 Ar ages derived from measurements on anorthoclase from pumice clasts, KBS Tuff, Koobi Fora Formation, Kenya

					34Ar/40Ar voes	us <sup>39</sup> Ar/ <sup>40</sup> Ar	
K-Ar <b>agc</b> (Myr) ±1 s d	**Ar/**Ar total fusion ago (Myr)±1 s.d	Incremental total fusion age (Myr) ± 1 s.d	Data used in regression	Plateau age (Myr) ; ±1 s d.	regree Age (Myr)±1 s.d.	mon (***Ar/;**Ar) <sub>1</sub> ±1 s d.	MSWD
			All data	$1.890 \pm 0.026$	1.860±0 011	$306.1 \pm 6.0$	26
	(1) $1900 \pm 0.014$	$1.890 \pm 0.026$	Exclude 700°, 770°	$1.881 \pm 0.023$	1 865±0 013	302.6±68	2.7
$189 \pm 002$			steps .				
	(2) —	$1.880 \pm 0.025$	All data	1.880 ± 0 025 1	$1.853 \pm 0.010$	$303.1 \pm 2.9$	1.9
•			Exclude 760° step	$1.875 \pm 0.024$	$1.874 \pm 0.013$	293.8±49	1.4
1 88±0 02	$1.881 \pm 0.011$	$1.879 \pm 0.027$	All data	$1.879 \pm 0.027$	$1.883 \pm 0.010$	296.0±20	19
			All data	$1.925 \pm 0.037$	$1.859 \pm 0.019$	$304-1\pm5.0$	10.3
1.88±0 02	$1.879 \pm 0.023$	1 925±0 037	Exclude 600°, 760°, 1,250° steps	1.893±0.034	$1.868 \pm 0.017$	289.3±5.0	64
	(Myr) ±1 s d 1 89 ±0 02 	(Myr) tusion age $\pm 1 \text{ s d}$ (Myr) $\pm 1 \text{ s.d}$ (1) 1 900 $\pm 0.014$ 1 89 $\pm 0.02$ (2) — 1 88 $\pm 0.02$ 1.881 $\pm 0.011$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	(Myr) fusion age fusion age Data used in regression  All data  (1) 1 900 ± 0.014 1.890 ± 0.026 Exclude 700°, 770° steps  (2) — 1 880 ± 0 025 All data Exclude 760° step 188 ± 0 02 1.881 ± 0 011 1 879 ± 0.027 All data  1.88 ± 0 02 1.879 ± 0.023 1 925 ± 0 037 Exclude 600°, 760°,	(Myr) fusion age fusion age (Myr)±1 s.d (Myr)±1 s.d in regression ±1 s.d.  All data 1890±0.026 (1) 1900±0.014 1.890±0.026 Exclude 700°, 770° 1.881±0.023  189±0 02 (2) — 1880±0 025 All data 1.880±0 025 Exclude 760° step 1.875±0.024  188±0 02 1.881±0 011 1879±0.027 All data 1.879±0.027  All data 1.925±0.037  1.88±0 02 1.879±0.023 1925±0 037 Exclude 600°, 760°, 1.893±0.034	K-Ar age (Myr) fusion age fusion age (Myr) ± 1 s.d fusion age (Myr) ±	(Myr)         fusion age         fusion age         Data used         (Myr)         Age         (*OAr/**Ar) <sub>1</sub> ±1sd         (Myr)±1s.d         (Myr)±1s.d         m regression         ±1sd.         (Myr)±1s.d         ±1sd.           All data         1890±0.026         1.860±0.011         306.1±6.0           (1) 1900±0.014         1.890±0.026         Exchde 700°, 770°         1.881±0.023         1.865±0.013         302.6±6.8           189±0.02         (2)         —         1.880±0.025         All data         1.880±0.025         1.853±0.010         303.1±2.9           Exchde 760° step         1.875±0.024         1.874±0.013         293.8±4.9           1.88±0.02         1.881±0.011         1.879±0.027         All data         1.879±0.027         1.883±0.010         296.0±2.0           1.88±0.02         1.879±0.023         1.925±0.037         1.859±0.019         304.1±5.0           1.88±0.02         1.879±0.023         1.925±0.037         1.868±0.017         289.3±5.0

 $\lambda = 5.543 \times 10^{-10} \text{ yr}^{-1}$ 

(40Ar/34Ar), refers to composition of mitial or trapped Ar

Incremental total fusion ages, calculated from all data obtained during a step heating experiment by combining the ages and errors according to the proportion of <sup>39</sup>Ar in each step, agree satisfactorily with the directly measured total fusion ages, as the errors overlap in all cases (Tables 1 and 2).

Age spectra are shown in Fig. 1, in which the calculated age for each step, together with its error, is plotted against fraction of <sup>39</sup>Ar released. The age spectra are similar to one another and are characterized by flat patterns or plateaus consisting of many steps which yielded essentially concordant ages over most of the release of 39Ar. Data are also shown on the 36Ar/40Ar versus  $^{39}\mathrm{Ar}/^{40}\mathrm{Ar}$  plot in Fig. 2. Table 2 summarizes the results. Plateau and regression ages are derived using all data from each step heating experiment, as well as by excluding results from steps that give discordant ages. The criterion for exclusion of a datum was that the calculated age differed by more than twice its error  $(2 \sigma)$  from that of the plateau. Generally the steps excluded account for only a small proportion of the gas release at the beginning or end of the experiment, and the differences between the results are small (Table 2). Overall the plateau ages and those derived from the regressions form a remarkably concordant set ranging from 1.85 to 1.92 Myr (Table 2).

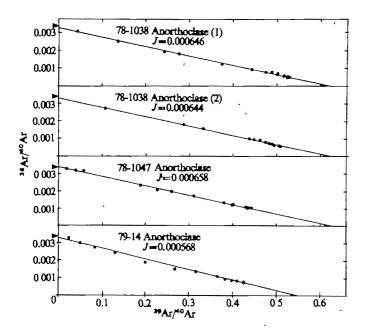


Fig. 2 Correlation diagram showing Ar isotopic composition for each gas fraction of each step heating experiment on anorthoclase from pulmice clasts in the KBS Tuff. A best fit straight line for each experiment is shown intercept on ordinate indicates composition of trapped Ar, and intercept on abscuras gives composition of the K-derived components, the inverse of which is proportional to age ., Isotopic composition of atmospheric Ar as midicated on ordinate

Two heating experiments were undertaken on anorthoclase 78-1038 to examine the reproducibility of an age spectrum. The two age spectra are similar (Fig. 1), each showing a welldeveloped plateau, the ages derived from which agree to within experimental error (Table 2). The second experiment yielded a virtually ideal plateau, comprising data from all steps except the first, which consisted of  $\sim 2\%$  of the total gas and which gave a high apparent age (Fig. 1). A similar effect is seen in the results from the first experiment, but the ages are only marginally high and are distributed over the first three gas fractions comprising ~5% of the <sup>39</sup>Ar. A possible explanation for the high ages for the early part of the release may be that some <sup>39</sup>Ar was lost by recoil from the marginal parts of the crystal fragments<sup>33</sup>. Results from the second experiment indicate that the gas fraction extracted at 1,160 °C, but lost in the first experiment, is unlikely to have given an age very different from those obtained for adjacent steps.

Regression of data from both step heating experiments on sample 78–1038 gave ages systematically slightly younger than the plateau ages (Table 2). This is reflected also in the <sup>40</sup>Ar/<sup>36</sup>Ar values derived for the initial or trapped Ar, the values for which are higher than the atmospheric Ar value of 295.5. These differences, however, are small and not statistically significant, especially when calculated after exclusion of the few discordant results (Table 2). There is greater scatter of the data points about the regression line than can be accounted for by experimental error in the first experiment, but for the second experiment the MSWD value is <2.5, so that all scatter can be attributed to experimental error.

The two anorthoclase samples from pumice clasts in the KBS Tuff correlative in area 131 also yield age spectra exhibiting good plateaus (Fig. 1). Sample 78-1047 has a virtually ideal flat spectrum and all ages derived from the combined data are concordant at 1.88 ± 0.01 Myr, irrespective of the manner by which they are calculated (Table 2). Gas from one step at 1,210 °C, comprising ~6% of the total 39Ar, was lost in the experiment on 78-1047, but it is reasonable to assume that this gas fraction would have given results similar to those obtained from adjacent steps. The other anorthoclase from area 131, sample 79-14, yielded a plateau for more than 85% of the release (Fig. 1), but shows high apparent ages for the first 5% of <sup>39</sup>Ar released and also for most of the last 8% of the release. The data points from this step heating experiment show significant scatter about the regression line, as indicated by the high value for the MSWD (Table 2). Even when the three most discordant points are excluded, there remains scatter in excess of that which can be accounted for by experimental error. If only those data are regressed that form the plateau between 5 and 92% of the 39Ar release, 'a line fitting to within experimental error is obtained (MSWD = 1.8). This regression yields an age of  $1.81 \pm 0.02$  Myr, and a  $^{40}$ Ar/ $^{36}$ Ar value for the trapped component of 362±7, which is statistically significantly different from the value for atmospheric Ar. Such a result conflicts with data from the other samples, and may be quite accidental because all the

points used in this regression plot are in a rather restricted portion of the correlation diagram with values for <sup>39</sup>Ar/<sup>40</sup>Ar between 0.31 and 0.43 (Fig. 2).

The flat 40 Ar/39 Ar age spectra over much of the gas release for anorthoclase from pumices in the KBS Tuff suggest that the distribution of <sup>40</sup>Ar\* in the crystals is relatively homogeneous. This conclusion would be correct if the effective dimension for <sup>40</sup>Ar\* diffusion (see ref. 34) is less than the size of the crystal fragments used in the experiments. Some of the anorthoclase crystals exhibit closely spaced (~2 µm) polysynthetic twins, so that it may be reasonable to propose that the distance between the twin planes is likely to be the effective dimension for 40Ar\* diffusion. Thus the lack of evidence for significant gradients of <sup>40</sup>Ar\* in the feldspar concentrates analysed is interpreted as indicating that closed system behaviour has prevailed in the feldspar since crystallization, at least in respect of the K-Ar

Regression analyses of data from the three anorthoclase samples yield a mean age of  $1.87 \pm 0.01$  Myr, and suggest that the composition of the trapped or initial Ar in the samples is indistinguishable from atmospheric Ar (Table 2). The plateau ages agree at  $1.89 \pm 0.02$  Myr, and the directly measured  $^{40}$ Ar/ $^{39}$ Ar total fusion ages average  $1.89 \pm 0.01$  Myr. An age of  $1.88 \pm 0.02$  Myr is suggested as the best estimate from the <sup>40</sup>Ar/<sup>39</sup>Ar data. This is in excellent agreement with the mean conventional K-Ar age of 1.89 ± 0.02 Myr derived from the results on seven anorthoclase samples analysed from pumices in the KBS Tuff in areas 105 and 131 (ref. 13). The concordancy of all these ages provides strong evidence that the anorthoclase has had a simple undisturbed history since crystallization from a rhyolitic melt. Geological evidence is interpreted<sup>3</sup> as indicating that deposition of the KBS Tuff occurred very soon after the explosive volcanism that produced the tuff and the pumice clasts, so that the measured ages are believed closely to approximate that of deposition. The simple geological history of the anorthoclase subsequent to its crystallization, incorporation in pumice clasts and deposition with the KBS Tuff, is consistent with the evidence that the tuff has never been covered by more than ~125 m of sediment, and with the weakly indurated nature of the sediments in the sequence.

Finally I comment on the previously published 11,16-19 <sup>40</sup>Ar/<sup>39</sup>Ar results on anorthoclase from pumices in the KBS Tuff. The  $^{40}\mathrm{Ar}/^{39}\mathrm{Ar}$  total fusion ages measured on 10 different concentrates, as summarized by Fitch et al. 19, range from 0.53 to 2.48 Myr, typically with quoted errors between 0.1 and 0.5 Myr. The proportion of 40 Ar\* in these analyses generally is <20% of the total <sup>40</sup>Ar and commonly <10% (ref. 18). On the basis of the large scatter in the ages and the small proportion of <sup>40</sup>Ar\* in the gas extracted from the anorthoclase concentrates, I suggest that the results are analytically less precise than given by these authors.

The results of <sup>40</sup>Ar/<sup>39</sup>Ar age measurements by the step heat-

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ing method on anorthoclase from KBS Tuff pumices, as reported by Fitch and coworkers 11,16-19, are difficult to evaluate. This is partly because analytical data from only one of the eight experiments have been published. In two other cases a form of age spectrum diagram was given, and several correlation diagrams were shown, but interpretations only are provided for some of the step heating experiments. The authors state that each age spectrum is complex. Ages derived from these spectra range from  $1.10 \pm 0.32$  to  $2.48 \pm 0.01$  Myr (refs 11, 18, 19). They interpret ages of ~2.48 Myr as reflecting crystallization of the anorthoclase, and the various younger apparent ages are attributed to subsequent thermal overprinting or disturbance of the feldspars. As noted previously13, geological evidence for such thermal events is lacking. I suggest that unrecognized analytical difficulties and larger than quoted errors must be invoked to explain these earlier 40Ar/39Ar results.

### **Conclusions**

The  $^{40}\mbox{Ar}/^{39}\mbox{Ar}$  age spectra on the three anorthoclase separates from pumice clasts in the KBS Tuff show well-developed plateaus over most of the gas release. This is interpreted as indicating absence of significant 40Ar\* diffusion profiles in the anorthoclase concentrates, consistent with the geological evidence that the samples are unlikely to have been thermally disturbed since crystallization. The 40 Ar/39 Ar total fusion ages, the plateau ages and those derived from the regressions are all concordant at 1.88 ± 0.02 Myr, in excellent agreement with our<sup>13</sup> earlier conventional K-Ar age measurements. As deposition of the tuff is considered to have occurred soon after explosive eruption of the volcanic material, this age provides a good estimate for the KBS Tuff, which must now be regarded as extremely well dated. Such an age is late Pliocene, as the Pliocene-Pleistocene boundary has an estimated age of 1.7 Myr (ref. 35). The precise, accurate dating of the KBS Tuff, a marker bed in the sedimentary sequence exposed adjacent to and east of Lake Turkana, also provides a similarly precise age for tuff bed H2 in the sequence in the Omo Valley, just north of Lake Turkana, as geochemical data<sup>20</sup> indicate that the two tuffs are likely to be equivalent.

Finally, our work confirms that the 40Ar/39Ar age spectrum method can be used successfully on Pliocene to Pleistocene rocks.

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# Early archaeological sites, hominid remains and traces of fire from Chesowanja, Kenya

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Recent investigations of Lower Pleistocene sites at Chesowanja have yielded in situ Oldowan and Oldowan-like stone artefacts, evidence of fire and a fragmentary 'robust' australopithecine cranium. Burnt clay found at one artefact locality dated to  $>1.42\pm0.07$  Myr is the earliest known evidence of fire associated with a hominid occupation site.

THE promise of the Pleistocene localities at Chesowanja, near Lake Baringo, was first shown in 1970, when a partial cranium of a 'robust' australopithecine was recovered<sup>1</sup>. Subsequent work by the late W. W. Bishop led to the recognition of early stone artefacts<sup>2-4</sup>, and systematic archaeological excavations began in 1978.

The exposed Lower Pleistocene sediments belong to the Chemoigut and Chesowanja Formations<sup>2,3</sup>. The Chesowanja basalt, dated to  $1.42 \pm 0.07$  Myr by K-Ar isotope dating<sup>5</sup>, is an important stratigraphic marker which immediately overlies the Chemoigut Formation and lies beneath the palaeosol member of the Chesowanja Formation.

### Archaeological sites in the Chemoigut Formation

The largest and most informative excavation in the Chemoigut Formation, GnJi 1/6E, was in the northern area (Fig. 1 inset), only 15 m from the most recently discovered hominid site. It covered 40 m², and nearly 1,000 artefacts were found in situ, together with numerous faunal remains, including some complete limb bones. Bovids, equids, hippopotamus and crocodile are all represented among the fossil bones recovered (a fuller report of the fauna will be published elsewhere).

The trench was cut in largely homogeneous compacted clayey silts. The sharply defined distribution of finds suggests that they accumulated in a silting-up channel, which can only otherwise be recognized by occasional grit lenses at its base. The finds have a limited vertical distribution (more than 50% occurring within 20 cm); there is no evidence of size sorting of either artefacts or bone and most of the finds occurred within the silts well above the gritty lenses, suggesting a low-energy environment of deposition. The fresh quality of the artefacts also suggests limited movement after discard. Alteration of the water level, perhaps due to seasonal factors, may have led to down-cutting. followed by infilling with almost identical sediments. This accords well with the view held by Bishop et al.2.3 that the localities represent sedimentation at the margins of a saline lake with a fluctuating water level. A similar abundance of antelope and crocodile remains has been noted in some of the Olduvai Bed I sites which have also been interpreted in terms of a lake margin environment<sup>6,7</sup>. The range of fauna, particularly the presence of hippopotamus and crocodile, suggests periods when the water was quite fresh<sup>7</sup>. Remarkable features in GnJi 1/6E were the flecks and lumps of red mineral matter scattered among the artefacts and bones (Fig. 2). A smaller trench, GnJi 1/5, about 100 m further north, also produced artefacts associated with a scatter of bones.

Excavations were also made in area 2 (GnJi 2) of the Chemoigut Formation, where hominid fossils were found in 1970 and 1974. Artefact scatters occur on the surface, but only one

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rewarding in situ occurrence, GnJi 2/8, was found. This horizon, which occurred only about 15 m from the hominid found in 1970, was close to the surface and could not be followed further laterally because of modern erosion gullies. Area 4 of the Chemoigut Formation contains at least one site (not yet investigated), but area 3 has yielded no finds.

### Sites in the Chesowanja Formation

The Chesowanja basalt is exposed to the east of the Chemoigut Formation. It plunges steeply to the east and is overlain by the palaeosol member of the Chesowanja Formation (Fig. 1, GnJi 10). Its upper surface slopes gently to the west and is sealed in by fine-grained bedded tuffs. These were mapped as belonging to the Karau Tuffs², which outcrop extensively to the west of Chesowanja, but further work has shown that they are a separate local unit, and hence as yet undated (A. Hill, personal communication).

Small artefacts were widespread on the surface of area 10, especially near exposures of the tuffs. Two trenches (GnJi 10/4 and 10/5) revealed dense concentrations of artefacts in the top  $50\,\mathrm{cm}$  of the Chesowanja Formation palaeosols, immediately underlying the tuffs.

In excavation GnJi 10/5, about 1,500 artefacts were found in situ in an area of 25 m<sup>2</sup>. Bone fragments included fish and crocodile remains.

The density of finds and the thickness of the horizon, which included lenses of sand stratified in the clayey palaeosol, suggest that the hominids returned to the locality over a considerable period. Some of the artefacts are abraded, probably due to prolonged surface weathering, possibly from physical transport by water. One such abraded artefact had been retrimmed freshly on one surface and is strong evidence for recurrent use of a site.

In area 10, the artefacts originally discovered in 1973 included Acheulean bifaces<sup>1-3</sup>. However, bifaces are absent from the *in situ* assemblages excavated in 1978, but have been found on the surface immediately above the bedded tuffs. Trial trenches demonstrated that a later channel had cut through the bedded tuffs, down into the Chesowanja Formation palaeosols. Bifaces were found on the surface of the channel fill, where one small specimen was recovered *in situ*. Thus the bifaces are clearly of much later date and should not be considered components of the Chesowanja Formation industry.

### Affinities of the stone industries

The assemblages from the Chemoigut Formation can be assigned to the Oldowan industrial complex<sup>6</sup>, in the broadest sense. We previously assigned surface collections to the Developed Oldowan<sup>4</sup>, but the finely shaped discoids which prompted this designation may be explained partly by the especially tractable nature of the fine-grained lavas used in stone-working. Such specimens are not common in situ and the general character of the artefacts is compatible with material from Olduvai Bed I/Lower Bed II<sup>6</sup> (Fig. 3). Thus it is appropriate to designate this

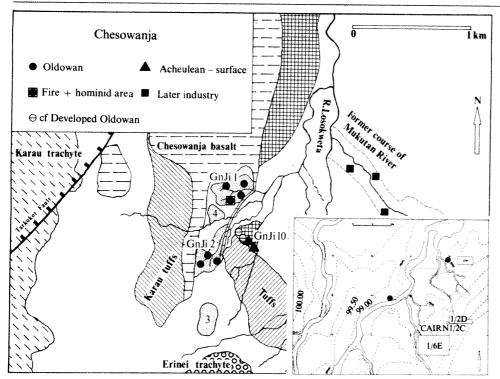


Fig. 1 Locality map of Chesowanja (compare with ref. 3, Fig. 20:1). 
Chemoigut Formation exposures; 
Chesowanja Formation palaeosol. Later sediments are not distinguished on the map. In the inset; 
, hominid remains; 
, modern gulley edges; ----, contours at 50-cm intervals. Scale, 10 m between crosses.

as the Chemoigut Industry within the Oldowan complex. One crude biface, made from an elongated cobble, was isolated on the surface of the Chemoigut Formation, but this is not insufficient basis for postulating the existence of a contemporary Acheulean facies. Finished tools from the Chemoigut Formation are on average slightly larger than those from the Olduvai<sup>6</sup> or Karari<sup>8</sup> industries, but the assemblage from GnJi 2/8 does include small forms. Technologically, the artefacts are broadly comparable with those found on the Karari sites, but they do not include the high-backed heavy-duty scrapers characteristic of the Karari Industry.

The industry from the Chesowanja Formation is also made mainly on lava, but some pieces are fashioned from welded tuff. The artefacts are strikingly small, and only 10 of more than 60 shaped tools weigh > 50 g. The size of the artefacts was

apparently not constrained by the availability of raw materials, for lava must have been abundant locally. Due to its distinctive characteristics and stratigraphic position, we define this material as the Losokweta Industry (after a local river) and in purely descriptive terms regard it as 'cf. Developed Oldowan'.

The new stratigraphic observations suggest a much later date for the Acheulean artefacts<sup>3,4</sup>. They seem to resemble artefacts from the Kapthurin Formation<sup>9</sup> more closely than those from sites in the Baringo basin such as Kilombe<sup>10</sup>.

### New hominid evidence from Chesowanja

Fragments of a hominid cranium, KNM-CH 304, were discovered in two separate scatters, 15 m apart (Fig. 1), by Bernard Ngeneo and Wambua Mangao in 1978. The specimen consists of five cranial vault fragments labelled A-E. A is a fragment of

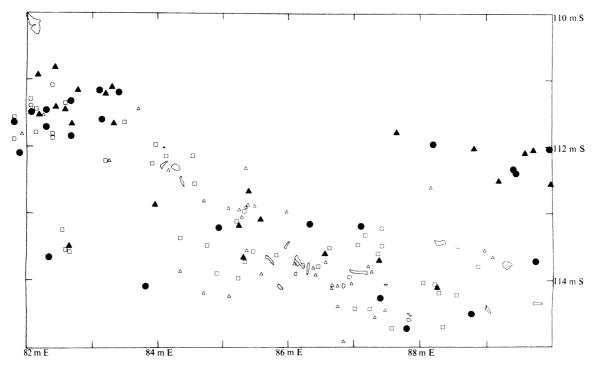


Fig. 2 Finds in trench GnJi 1/6E (excluding flakes and flaking debris). ●, Heavy-duty tools; ▲, scrapers (light duty); △, bones; □, burnt clay. Axes are scaled in metres.

Table 1 Arteract counts from Chesowanja determined by J. W. K. H. according to the typology used at Koobs Fora

	Chemoign 1/6E	it Industry .2/8	Losokweta Industry 10/5
Area excavated (m²)	40	16	25
Tools Choppers Polyhodrons	9	2 2	4 3
Discoids Partial discoids Scrapers Sundry	3 5 22	4 — 1 —	19 2 29 3
Total	47	9	60
Modified/edge damaged Debitage	28 855	5 126	25 1,411
Total artefacts	930	140	1,496
Unmodified cobbles/split cobbles	13	5	23

occipital which includes lambda, inion and most of the left side of the occipital squame up to, and including, asterion; the surface bone is cracked and eroded. B is the left parietal including bregma, part of the coronal suture and a length of sagittal suture and parasagittal crest; C is the left temporal squame; D the right parietal including pterion and part of the squamosal suture; and E a small fragment of right parietal. Table 2 gives the length and thickness of A, B and relevant comparative material.

The most obvious diagnostic features of the cranium are ectocranial crests. The fused sagittal crest is 3 mm high at bregma, but 30 mm posteriorly it splits into two parasagittal crests which, at their highest point, project 11 mm from the vault surface. They continue onto the occipital bone where, about 20 mm posterior to lambda, they each turn abruptly laterally and fuse quite close to the midline with the nuchal crest to form a compound nuchal crest. The nuchal crest on the left side divides near to asterion and the inferior limb fuses with a separate crest running along the occipitotemporal suture. There is a small 'bare area' between inion and the point where the parasagittal crests diverge. Other features useful for taxonomic diagnosis are a series of coarse ridges and furrows which run anterolaterally from the sagittal suture in the direction of pterion; fine striae which mark the parietal bone just superior to the squamous suture and a pattern of venous sinus grooves on the endocranial surface of the occipital fragment, suggests that the main drainage pathway for venous blood was via an occipital venous sinus.

The combination of an anteriorly situated sagittal crest and a compound nuchal crest is shared with only one other fossil cranium, OH5, the type specimen of Australopithecus bolsei<sup>11</sup> from Olduvai Gorge Tanzania. In another adult cranium belonging to the same taxon, KNM-ER 406, there is a substantial sagittal crest, but apparently no compound nuchal crest. In a juvenile cranium, L338y-6, from the Omo, the inferior temporal and superior nuchal lines nearly touch the left side whereas the inferior temporal lines are still 12 mm apart some 27 mm posterior to bregma<sup>12</sup>. Thus, if this individual had reached adulthood, a substantial compound nuchal crest would probably have been present. The adult skull KNM-ER 1805 from Koobi Fora, which still awaits formal attribution to a taxon, combines a compound nuchal crest with a sagittal crest, but in this case the sagittal crest is situated more posteriorly. It begins 30 mm posterior to bregma, and at stephanion the distance between the inferior temporal lines is at least 31 mm. The cranial vault morphology of fossils attributed to Australopithecus africanus suggests that sagittal crests may have occurred in some specimens, but there is no evidence that any of these crania had developed a compound nuchal creet.

The coarse ridges which mark the parietal are like those described by Tobias<sup>11</sup> in OH5, and similar features are also seen in KNM-ER 406. Finer striae—striae parietalis—are also

present on OH5, KNM-ER 406 and L338y-6. Rak<sup>13</sup> has carefully analysed these markings and he relates them to a special type of overlapping squamosal suture which has only been found in crania attributed to A. bolsei. The pattern of venous sinus grooves, with a dominant occipital sinus, resembles that seen in OH5, SK 859 and SK 1585<sup>14</sup>, and a similar pattern is suggested by the preserved morphology in SK 46<sup>15</sup>. However, at least one East African 'robust' australopithecine specimen, L338y-6, has the more usual human pattern<sup>12</sup> (but Holloway<sup>16</sup> is cautious about attributing this specimen to A. bolsei). Occipital sinus dominance is therefore best considered indicative, rather than diagnostic, of A. bolsei.

Thus, although the new cranium is fragmentary, sufficient diagnostic areas are preserved to attribute it with some confidence to A. bolsei, thereby confirming the presence of this hominid at Chesowania.

This specimen fails to provide any new support for the proposal of Carney et al. that the only other cranium known from Chesowanja, KNM-CH 1, represented an 'evolved' form of 'robust' australopithecine. Of the three possible features cited by these authors—a larger brain, a more flexed skull base and the reduction or refinement of masticatory apparatus—the new specimen is only complete enough to provide information about the last. The ectocranial crests suggests that the masticatory

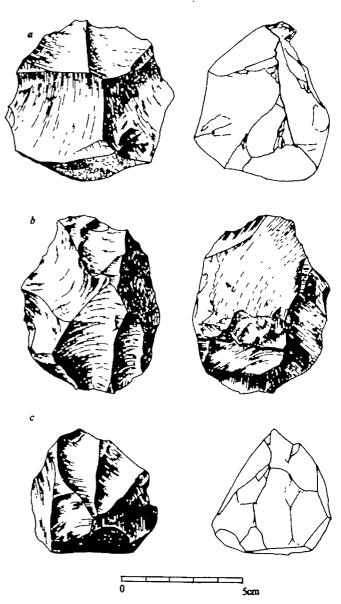


Fig. 3 Artefacts from the Chemoigut Formation. a, Chopper, b, discoid; c, polyhedron.

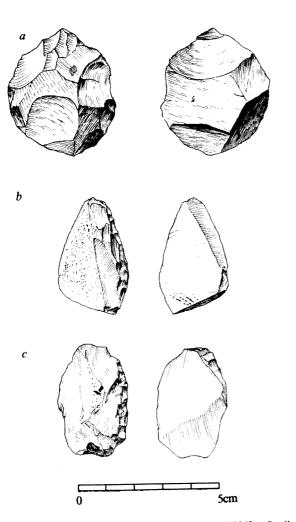
muscles in KNM-CH 304 were at least as well developed as they were in the two much better preserved 'robust' australopithecine crania from East Africa, KNM-ER 406 and OH5.

### Evidence and implications of fire

The possession of fire is a major component of cultural behaviour, and is universal in recent times even among hunters and gatherers. Evidence for fire is relatively common on later Acheulean sites<sup>17</sup> ( $\sim 0.2$ –0.3 Myr), but rare in earlier sites; Vertesszöllos<sup>18</sup> (Hungary), Arago<sup>19</sup> (southern France) and Choukoutien<sup>17</sup> (China) are the main examples and none of these is believed to be older than 0.5 Myr.

If, however, fire had been used on earlier sites, evidence for it might have vanished. Isaac<sup>20</sup> noted that the fine charcoal remaining from wood fires is easily degraded in tropical conditions and readily removed by wind and water. Thus on open sites, baked material is likely to provide the best evidence of fire. Inconclusive evidence for fire has come from magnetic and thermoluminescence (TL) analysis of material from reddened areas of ground at site FxJj 20 East<sup>21</sup> on the Karari escarpment at Koobi Fora (~1.3-1.5 Myr) and from palaeomagnetic determinations on cobbles at an Acheulean site at Gadeb in Ethiopia<sup>22</sup>.

At Chesowanja, over 40 pieces of 'burnt clay' were found on the GnJi 1/6E site. They range in size from tiny flecks to lumps 5-7 cm across. They were thoroughly intermingled with the artefacts and bones (Fig. 2) and did not occur without this association. They could not have been introduced into the site after its formation. Samples of the supposed burnt material were



**Fig. 4** Artefacts from the Chesowanja Formation, site GnJi 10/5. a, Small discoid; b c, scrapers.

Table 2 Measurements of KNM-CH 304 and relevant comparative material KNM-CH 304 OH5 KNM-ER 406 Parietal Sagittal chord 80 76 Thickness at bregma Thickness at asterion Occipital Inion-left asterion chord 52 38 Lambda-inion chord Thickness at lambda 10 7.0 Thickness at asterion 14.5 7.0

All measurements are in mm and, except those marked\* taken from ref. 11, were taken by BAW

examined by one of us (D.W.). One sample possessed a magnetic moment of the order of  $10^{-4}\,\mathrm{A\,m^2\,kg^{-1}}$ , which is broadly comparable with the magnetic moment of more recent samples of burnt clay  $^{23,24}$ . On heating and cooling in zero magnetic field, the moment disappeared at  $400\,\mathrm{^{\circ}C}$ —a normal temperature for open camp fires, which seldom exceed  $700\,\mathrm{^{\circ}C^{25}}$ .

Alternatively, the baked material could have resulted, not from a controlled camp fire, but from a 'wild' bush fire. However, although these create transient surface temperatures of up to 700 °C, the temperature then falls away rapidly below the surface<sup>26</sup>, so that baking of clay would be unusual. Investigations of baking around a recently burned tree stump near Chesowanja showed that the temperature was at least 200 °C higher than that of the fire at GnJi 1/6E<sup>21</sup>.

Thus, although the magnetic evidence is not in itself conclusive, it nonetheless strongly suggests that the Chesowanja clay was burned by a small, controlled fire. Future studies of the cooling rate may provide more specific evidence about its size. A possible explanation for the distribution of the detached lumps of burnt clay is provided by analogy with a later prehistoric hearth observed by one of us (J.A.J.G.) at Kilombe. A fire had baked the underlying sediments, which are now being removed by modern erosion. The burnt clay, being more resistant, was raised slightly above its surroundings, but was cracking and breaking up, and lumps of it were scattered for several metres downslope from the source.

Although natural phenomena such as bush fires, lightning strikes and even volcanic heating could explain the burnt clay at Chesowanja, we are convinced, from examination of the whole occurrence in situ, that hominid activity is a much more likely explanation. Thus, the new find, together with the more tentative evidence from other sites, greatly strengthens the hypothesis that by 1.4 Myr hominids were using and controlling fire.

### **Conclusions**

At Chesowanja hominid remains of robust australopithecines have now been found close to archaeological finds at two separate localities almost 1 km apart. This association must be considered more than coincidental. At both sites non-hominid bones and bone fragments are closely associated with the artefacts, and it is likely that the artefact makers also consumed the meat.

The detailed dietary preferences of these robust australopithecines are not known, but preliminary evidence from studies of tooth microwear suggests that meat was not a significant component of their diet<sup>27</sup>. The Chemoigut Formation sites are approximately contemporary with early specimens of *Homo erectus* found elsewhere in Africa<sup>28</sup>. H. erectus was apparently responsible for the use of fire at Choukoutien and the use of fire by early representatives of the same hominid type at Chesowanja seems to us the most reasonable hypothesis. The principal alternative would involve the robust australopithecines being not merely meat-eaters, but also stone-tool makers and fire-users; we believe that the evidence is strongly against such a view.

If we accept the former hypothesis, then the presence of robust australopithecine fossils at both of these localities is difficult to explain on any basis except a chance association, or that their remains were brought to the site, along with those of other animals, by the makers of the artefacts.

The paucity of evidence bearing on these hypotheses underlines the necessity of locating and investigating new sites of the same period where there is well preserved and in situ evidence of hominid occupation

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### Structure of the pro $\alpha 2(I)$ collagen gene

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Fifty-four kilobase pairs (kbp) of cloned chicken DNA containing the entire 38-kbp pro  $\alpha 2(I)$  collagen gene have been isolated and characterized. DNA sequence analysis of a select 4 kbp of the gene has precisely described 14 exons which comprise one-third of the sequences encoding the triple-helical domain of the collagen protein. These exons range in size from 45 to 108 base pairs (bp), are all multiples of the 9 bp that code for the repeating triplet, Gly-X-Y, and have an average size of 70 bp. About 50 introns interrupt this gene. Nevertheless, introns do not separate the coding sequences for the ends of the central triple-helical structural domain and the ends of the propeptide domains.

THE collagens are a set of closely related proteins which provide the extracellular framework responsible for the structural integrity of nearly every organ and tissue in vertebrates. The correctly programmed spatial and temporal regulation of the production of collagen molecules is critical for growth and development. Therefore, the genes which encode these strategically important molecules deserve detailed investigation. Our investigations and those elsewhere have concentrated on the genes which encode the two chains that make up type I collagen, the most abundant of the collagen family.

The major part of the mature collagen consists of three polypeptide chains, called  $\alpha$ -chains, arranged in a long rod-like helical configuration. This region contains about 338 repeats of the amino acid triplet Gly-X-Y, where X and Y are often proline and hydroxyproline. Each polypeptide chain is synthesized as a propeptide with nonhelical regions at both the amino- and carboxy-terminal ends; the propeptides are removed by specific endopeptidases, leaving much shorter nonhelical regions, termed telopeptides, attached to both ends of the collagen helix. Five different types of collagen have been identified: they are made up from nine  $\alpha$ -chains which thereby require nine different genes1.

Recombinant DNA technology has made possible the isolation and structural analysis of several dozen unique eukaryotic genes. With the exception of the interferon genes, all unique genes in vertebrates have been found to contain noncoding sequences (introns) which interrupt their coding sequences (exons). The number of introns ranges from a single intron, as in the rat insulin I gene<sup>3</sup>, to many introns, as in the Xenopus laevis vitellogenin gene which has 33 (ref. 4).

Our preliminary studies of the structure of the chicken pro  $\alpha 1(I)$  and pro  $\alpha 2(I)$  collagen genes, using actinomycin D/CsCl gradients and genomic DNA Southern blotting techniques, indicated that both these collagen genes would contain many introns5. This was dramatically confirmed for both the sheep and chicken pro  $\alpha$ 2 collagen genes by electron microscopy studies of gene clones hybridized to procollagen mRNA. Almost 40 introns, ranging in size from <100 bp to >2,000 bp, could be clearly identified in the electron micrographs of gene clones spanning the entire chicken pro \alpha 2 gene reported by Ohkubo et al.<sup>6</sup>. Seventeen introns were similarly identified in gene clones containing 60% of the sheep pro  $\alpha 2$  gene<sup>7</sup>. Thus, the pro  $\alpha 2$ collagen gene has emerged as the most highly interrupted gene so far examined.

To investigate the intron-exon structure with precision, extensive sequence determinations are required. We have reported the DNA sequence analysis of seven exons in the helical region, ranging in size from 45 to 108 bp8. All are multiples of 9 bp coding for the repeating Gly-X-Y collagen triplet and each begins with the codon for glycine and ends with the codon for the Y residue. The exon sequences reported by Yamada et al.9 have this same characteristic. However, the structure of the carboxy-terminal propeptide is quite different: we found the four exons there to be much larger, ranging in size from 189 to 444 bp8.

We report here the detailed structural analysis of 4 kbp of the collagen gene including the DNA sequence at both the amino-

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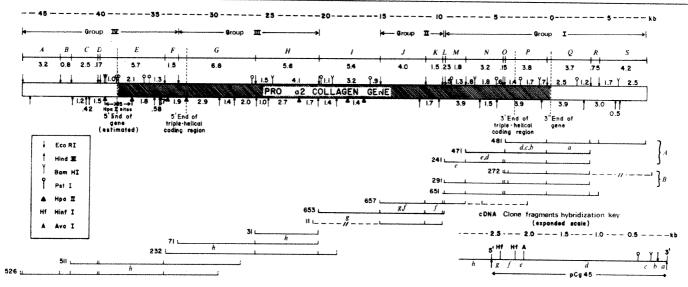


Fig. 1 Restriction endonuclease map of pro α2 collagen gene clones from chicken (Gallas domesticus). Clones containing EcoRI fragments I-S were isolated by hybridization to the cDNA clone pCg45 (ref. 25), those containing fragments G and H by cross-hybridization to pCg45 and those containing fragments A-F by hybridization to the EcoRI-HpaII fragment at the 5' end of EcoRI fragment G. As described previously<sup>8</sup>, the location and orientation of each of the clones were determined by hybridization of the clones digested with various restriction endonucleases to nick-translated fragments of pCg45 labelled a to g in the hybridization key. Fragment h refers to hybridization to kinased procollagen mRNA sequences not contained in pCg45. Vertical lines in the individual clones indicate EcoRI sites; dotted vertical lines indicate synthetic EcoRI sites generated by the linker molecules used in the construction of this λ library<sup>26</sup>.

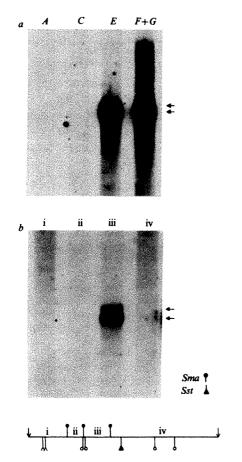


Fig. 2 Location of the 5' end of pro  $\alpha$ 2 collagen gene. Calvaria mRNA was isolated, glyoxylated, electrophoresed on 1% agarose gels and blotted onto nitrocellulose as described by Thomas<sup>27</sup>. Isolated fragments were nicktranslated and hybridized to lanes cut from the RNA blots. a, The EcoRI fragments used as probes are indicated above each lane (see Fig. 1 for locations of fragments). Arrows indicate positions of pro  $\alpha$ 2 collagen mRNAs, located by hybridization to cDNA clone pCg45. The blot was overexposed to confirm lack of coding sequences in EcoRI A and C. The origin of the weaker, upper band is undefined, but may represent alternatively processed molecules or a stable precursor. b Shows hybridization to isolated subfragments of EcoRI E, the origins of which are shown below in the restriction endonuclease map of the fragment. Symbols as in Fig. 1. Hybridization to the Smal-EcoRI subfragment iv is extremely weak, suggesting that there is little coding sequence in this fragment.

and carboxy-terminal junctions of the helical and propeptide coding regions of the gene, the DNA sequence of seven exons near the central portion of the gene which code for contiguous parts of the protein (residues 175-360) and the location, by DNA sequence analysis, of four other exons coding for residues 4-72.

### Isolation of DNA fragments

We have previously reported the isolation of recombinant A clones containing  $\sim 80\%$  of the pro  $\alpha 2(I)$  collagen gene, and have described in detail the structure of the 3' portion of this gene8. We have now isolated overlapping clones which can be arranged into a 54-kbp piece of contiguous genomic DNA containing the entire pro  $\alpha$ 2 collagen gene. Figure 1 shows a partial restriction map of this DNA region, along with some of the  $\lambda$  clones which together span the gene. The cloning strategy is detailed in the Fig. 1 legend. The 5' end of the gene was located at the position indicated in Fig. 1 by two independent methods. Hybridization of labelled EcoRI fragments from the gene clones to procollagen mRNA indicated that the 5' end was probably located in fragment E, because positive hybridization was not obtained with any fragment lying 5' to this region (Fig. 2a and unpublished observations). Subdivision of fragment E with restriction enzymes and the use of these fragments to probe blots of procollagen mRNA enables us to locate the 5' end of the gene (Fig. 2b). These experiments do not rigorously exclude the possibility that there is another exon lying >8.3 kbp 5' to this region, nor that RNA transcription initiates at some point 5' and is so rapidly processed that it is not detected. However, the placement of the 5' end of the gene in this region is consistent with the results of in vitro RNA transcription using truncated gene clones as templates in the Manley 10 cell-free extract system (V.T., unpublished observations). This result, together with the hybridization experiments, place the 5' end of the pro  $\alpha$ 2 collagen gene at the point indicated in Fig. 1. The 5 kbp of coding sequence for the pro  $\alpha$ 2 collagen mRNA is thus spread over a total of 38 kbp of genomic DNA.

#### Triple-helical coding sequences

Although electron microscope studies of mRNA-DNA hybrids may provide an overall picture of exon-intron arrangements in the pro  $\alpha 2$  collagen gene, only DNA sequence analysis can establish the exact size and coding capacity of the exons and the precise location of specific coding regions in the gene. As the

coding information of the gene is so dilute, it is necessary to locate DNA fragments which contain coding information in order to concentrate DNA sequence analysis where it is most valuable. Such regions were identified by hybridization to cloned cDNA or kinased procollagen mRNA and the locations determined more precisely by the use of restriction enzymes which cut sequences predicted to be abundant in the collagen triple-helical region but relatively infrequent elsewhere (see Fig. 4 legend).

The DNA sequences from the 5' end, the 3' end and central portions of the helical region of the pro  $\alpha$ 2 collagen gene were then determined. Fifteen exons, including seven reported previously8, have been located (Fig. 3). The residue numbers of the amino acids in the triple-helical region coded for by each exon are given below. Figure 4 shows the DNA sequence of the genomic region containing the exons coding for residues 175-360 of the triple-helical region. Figure 4a displays the entire sequence of the 1.8-kbp HpaII-EcoRI fragment located at the 3' end of EcoRI fragment H (Fig. 1), while the sequencing scheme used is shown below. This fragment is seen to contain five exons which together encode residues 175-309. Figure 4b displays the DNA sequence of a region of EcoRI fragment I together with the sequencing scheme. Two exons are indicated, coding for residues 310-360. The seven exons contained within the sequences given in Fig. 4 thus code for contiguous parts of the protein (these exons are numbered 29-35 in Fig. 6.)

The DNA sequence determination of 14 complete exons coding for the triple-helical region has further detailed the remarkable structure of the pro  $\alpha 2$  collagen gene: it contains a very large number of exons, each with a correspondingly small amount of coding information; each exon is a multiple of the 9 bp which code for the repeating collagen triplet (Gly-X-Y). Of these 14 exons, 2 contain 45 bp, 7 54 bp, 3 99 bp and 2 108 bp. Although the most frequent exon size is 54 bp, it represents a total of only 7 out of the 14 exons. This contrasts with previous reports that all the exons in this gene contain 54 bp.

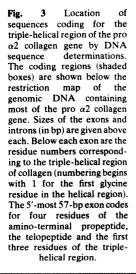
The seven exons coding for residues 175-360 of the triplehelical region show an interesting alternation between large and small exons: 99-45-99-54-108-54-99. This pattern may be extended by including sequence determinations of two additional exons by Yamada et al.9. Exons coding for residues 379-411 (99 bp) and 412-429 (54 bp) were found; an exon of 54 bp, coding for 361-378, therefore almost certainly exists between these two exons and the seven exons whose sequence is given in Fig. 4. This would extend the alternating pattern to 10 exons, coding for 25% of the helical region. Such a pattern may well also exist in the 3' portion of the molecule, but it is clearly absent in the 5' end of the helical coding region of the gene. There is no obvious explanation for this alternating pattern nor why exons of other multiples of 9 bp are not found. Structural domains within the  $\alpha$ 2 triple-helical region of sizes D (234 residues), D/6 (39 residues), D/11 (21 residues) and D/13 (18 residues) have been proposed on the basis of intra-chain amino acid sequence comparisons<sup>11</sup>. Although the latter domain size is the same as the most prevalent exon size, the fact that about half the exons encode 15, 33 or 36 residues indicates that there is little correlation between the exons and structural domains in the helical region.

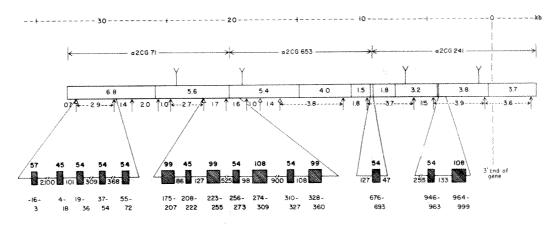
The finding that the triple-helical coding region of the pro  $\alpha 2$ collagen gene contains many small exons, each encoding a multiple of the Gly-X-Y repeat, suggests that the gene may have evolved by duplication. As seven of the eight exons first sequenced were reported to be 54 bp long, Yamada and coworkers proposed that the ancestral collagen gene was 54 bp and that during evolution this gene was duplicated to give rise to the present structure9. In this scheme, the large present-day collagen gene was formed by recombination between introns, followed by additions and deletions of 9 bp. The fact that only 7 of the 14 exons reported here are of 54 bp suggests a different mode of evolution for the collagen gene. If an ancestral triplehelical region gene of 54 bp is assumed, duplication by recombination between intergenic regions or introns (depending on the presence or absence of the propeptide coding regions) could indeed give rise to several 54 bp units. However, inasmuch as introns appear to be free to diverge rapidly, as compared with coding regions, recombination between different introns would be expected to be infrequent. Therefore, the repeating nature of collagen would make homologous recombination between different exons a far more likely event. We suggest that it is most likely that duplication took place in large part by recombination between exons (homologous unequal crossing over) rather than between introns. At each recombination point the exon size could change, readily accounting for exons differing from 54 bp. Recombination between two exons at non-identical positions within the 9 bp coding for the Gly-X-Y repeat would result in disruption of the helical structure of collagen, and thus be deleterious or fatal. Therefore, the exon sizes would always be maintained as a multiple of 9 bp.

### Structural domain junctions

The DNA sequences of the exons coding for both the aminoand carboxy-terminal ends of the triple-helical region have also been determined. In both cases, these exons also code for the telopeptides and parts of the propeptides (Fig. 5). The exon at the amino-terminal end codes for the last 4 residues of the propeptide, all 12 residues of the telopeptide and the first 3 residues of the triple-helical region.

Although the amino acid sequence of the chicken aminoterminal propeptide has not been determined, this sequence is known for calf pro  $\alpha 1$  collagen<sup>12</sup>. The amino acid sequence around the amino-terminal propeptidase cleavage site for pro  $\alpha 1$  collagen is Asn-Phe-Ala-Pro $\downarrow$ Gln. The DNA sequence of the amino-terminal junction exon (Fig. 5) yields a similar





 ${\tt GGGCCATTCTGTGATTCTGTGTGATTATAATGTCAGGCTAATGAAGTTACTCTGCAATTGCCCTAACACTCTGGATACTGATACAGAGCTTTATCCTTTTCTCAAACAGGGTGAAATCGG\\ GlyCluIleGl$ 

121 ACCTGCTGGTAATGAAGGCCCTACTGGTCCTGCTGGTCCAAGAGGAGAGTTGGACTTCCTGGTTCCAGTGGTCCTGTTGGCCCTCCCGTGAGTACCTTGCCAGTCTTTATCTATTGTCA yProAlaGlyAsnGluGlyProThrGlyProAlaGlyProArgGlyGluIleGlyLeuProGlySerSerGlyProValGlyProPro

241
GATGGATGCATTTGAGACTGAATTAACCAGTATAATTCTTTTCTGCCTCCATAGGGCAACCCTGGTGCTAATGGTCTTCCTGGGGCTAAAGGTGCAGCTGTAAGTATAGCCACATGCACA
GlyAsnProGlyAlaAsnGlyLeuProGlyAlaLysGlyAlaAsla

481 TGCTGGTGCTCCTGGTCTGCCTGGGCCCCGTGGTATTCCTGGTCCTCCTGGCCCTGCTGGTCCAAGTGGTGCTAGGGGACTTGTTGTAAGTGACTTTGTTTACTGCATGTCTTCATTTGA lAlaGlyAlaProGlyLeuProGlyProArgGlyIleProGlyProProGlyProAlaGlyProSerGlyAlaArgGlyLeuVal

601 AAACTGGATACAGCATCCATCATTGTAAAATATTTCTTCTAGATATGTTCCTTCATTGAATGCAGACACTTTTGTTGCTTCAGTATAATTAAGCATTGTGAGCTTGTTTCTGTCTCTCAG

721 TGAGACTGAAGAGGTTCACTTTGTACTCCCTTTGGCTGCAAGAACTATTAAGCTCTGACCCTCTTGATGGTATCAACAGAGATGTGACAGCAGTCTCACAATCAGAACCTCAAGGGGGTAT

961 ATCAAAATAGAGTCAGGCATGACACTGGATGATTAGAGATGGTTGTGATTCATGATAATGTTGTACAGGTAGAAGATCTGTAGAATGGTGCTTAATTCTAGAATCTCTTTTTATGT

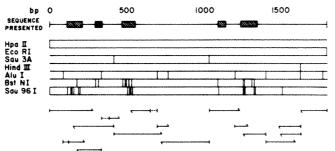
1081 TGGACTCTAGGGTGAACCAGGCCCTGCTGGTGCCAAGGGGAGAAAGTGGTAACAAGGGTGAGCCTGTAAGTATGGCAATTTGTGAGTGTTTCAATGAATATAGTCTCATAGTACCTAG GlyGluProGlyProAlaGlyAlaLysGlyGluSerGlyAsnLysGlyGluPro

 ${\bf 1201}\\ {\bf TAGCTCAGCTCTAATTTATTCTTTTCCTCTTGGCTGAGGTAGGGTGCTGCTGGCCCCCCTGGCCCTCCTGGTCCAAGTGGTGAGGAAGGCAAGAGAGGGCAGCAATGGTGAACCTGGCTCT\\ GlyAlaAlaGiyProProGlyProSerGlyGluGluGlyLysArgGlySerAsmGlyGluProGlySer\\ {\bf 201}$ 

1441 ATGACTACATTTGTGTTAATGCAACTAGGCAAAGGTCATTTTGCTGTTCTGTGCCTTTTGATTTGCTGGGACCAACCCTATTTTTGTTTTGCATTAATGATGTTTATTATCTTATCCCTG

1561 AATTAATTTAAGGTTGCTTTGTGTTATCCACTGATTAATATTTAGAGAAATAGCACTAACTTTGCTGTGAAGCTTGAGATATAAGTGCTCGAAAGAGATAGTTGGCTCACATGTAATGTT

TGGGAGAGGTTTTAAGTTCAGTGCCTTCGAGGGTATCTGTAGTACGCTTATCACAGGGCGAGGAACAACACAATGTCTTTCAGGAGGCACGTAGCTTTTGAGCAGGTGCAGGTTTAGGAA



1 AACOGGAACAGAGTTTTCTTTCCTTTTCAGATTTAATTTGTATTCACTTGCAGGGCGAGGCCTGGATCTCGTGGTCTCCCTGGAGCTGATGGCAGAGCTGGTGTCATGGTAAGGCTTGTC GlyGluProGlySerArgGlyLeuProGlyAlaAepGlyArgAlaGlyValMet

AGTOGACCTGTTGGTGCTAAGGGTCCTAATGGTGATGCTGGCCGTCCTGGTGAACCTGGTCTTATGGGTCCAAGAGTAAGTCTAGAACTGTGCAAAAAGAAACTACTTGTATTTGTGGTA SerGlyProValGlyAlaLyeGlyProAenGlyAepAlaGlyArgProGlyGluProGlyLeuMetGlyProArg

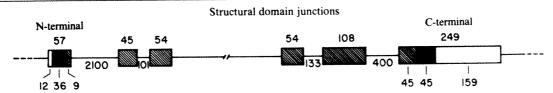
361 AATTATCAGAAATCCAATTGCAGGAAGTTCTATTCAAGCCACAATTAGACTGTTTCTATCAGCCCACTGAAAAAACATCCGTGAGGGAATGATTAAGCAGCATCAAAATGTTTATTGAAA

### 481 TTCATTTCTTTAGTAATCTGGTGGCATCTAATTGCCTTGGCCATGG

Fig. 4 a, DNA sequence of the 1.8-kbp Hpall-EcoRI fragment at the 3' end of the EcoRI fragment H (coding for residues 175-309). b, DNA sequence of part of the 2.4-kbp *EcoRI-HpaII* fragment at the 5' end of the *EcoRI* fragment 1 (coding for residues 310-360). The sequencing schemes are shown below the DNA sequences. DNA sequences



were determined by the method of Maxam and Gilbert<sup>28</sup>. Triple-helical coding sequences were located by restriction of isolated gene fragments with *Bst*NI (an isoschizomer of *Eco*RII) and *Sau*96l. From the DNA sequences of the pro a2 collagen triple-helical region determined from the cDNA clones, many of the Pro-Gly sequences of collagen will contain BstNI sites, CC(A/T)GG. Sau961 recognizes the sequence GGNCC, which is also predicted to be very common in the triple-helical coding regions as collagen has many Gly-Pro sequences. The BstNI and Sau961 sites identified by DNA sequence determinations are indicated on the last two lines of the restriction map in a. All 13 BsfNI sites in this 1.8-kb fragment of DNA can be seen to fall within the collagen coding sequences (indicated by the shaded boxed above), while 16 of the 18 Sau961 sites also lie within coding regions. Thus, the correlation of these sites with triple-helical coding regions is excellent. The amino acid sequence for residues 175-327 of chicken  $\alpha$ 2 collagen has not been determined; however, the calf collagen sequence is known<sup>29</sup>. Of the 153 residues determined by DNA sequence analysis here, 133 are identical to the calf sequence, indicating an 87% homology. Only one instance was noted where the DNA-derived amino acid sequence analysis nere, 133 are identical to the can sequence, indicating an 67% nonlongly. Only one instance was noted where the DNA-derived amino acid sequence differs from that determined by amino acid sequencing of chicken  $\alpha 2$  collagen. The DNA sequence indicates that residues 339 and 341 are valine and alanine, whereas the reverse was found by amino acid sequencing. Although it is possible that this represents an allelic difference, the discrepancy may be due to an error in the amino acid sequence determination, as the lack of a thermolysin site which should exist preceding the valine residue was noted when the protein sequence was presented.



N-terminal:

1 ACCCTTCCTACTGAATCAAGTCTTTCTGGAAACAGATGAATAGAAATGTATTTGTAAAAGGGTCATGTGTACTGAGTTTTAAGTATGTTGCTTCAACATACTGTCTAGCAAATGAGGTAA
121
TGGATACATGTAGAATGAGGGGTTATCAGTGCTTTCTGAAGTAAGAATTGTAGTGACTTTTATTTCAAATTTTTGTCATCTACAGAATTTTGCTGCTCAGTATGATCCATCTAAAGCGGGCT
AsnPheAlaAlaGlnTyrAspProSerLysAlaAla

 ${\tt 241}\\ {\tt GACTITGGCCCCGGACCTATGGTAAGTATATGATTTAACACTTGGTAACTTGCATAAACATGTATTTAAGTGTACTCCAG}\\ {\tt AspPhsGlyProGlyProMet}\\$ 

C-terminal:

121
CCCCTGGCCCCCTGGTCCCAATGGTGGCGGATATGAAGTTGGCTTTGATGCAGAATACTACCGGGCTGATCAGCCTTCTCTCAGACCCAAGGATTATGAAGTTGATGCCACTCTGAAAA
roprofiuproprofiuproAsnGluCluGluTurGluValGluPheAspAlaGluTurTurArgAlaAspGlnProSerLsuAraProLusAspTurGluValAspAlaThrLsuLusT

241
CATTGAACAACCAAATTGAGACCCTGCTGACCCCAGAAGGCTCCAAAAAGAACCCGGCTCGCACCTGCCGTGACCTCAGACTTAGCCACCCAGAATGGAGCAGCGGTACGTGGTGCCAGA
hrleuhenhenGinIleGluThrleuleuThrProGluGlySerLyelyehenProAlaArgThrCyehrghepLeuhrgLeuserHieProGluTrpSerSerGly

361 TOTTTCCTCTTTCTGGCTCAGTAAGTCATTTTCA

Fig. 5 DNA sequences of the genomic regions containing the exons coding for the ends of the helical region. Propeptide coding regions are indicated by open boxes, telopeptide coding regions by solid boxes and triple-helical coding regions by shaded boxes. The amino-terminal junction exon codes for the last 4 residues of the propeptide, all 12 residues of the telopeptide and residues 1–3 of the helical region. The carboxy-terminal junction exon codes for residues 1,000–1,014 of the helical region, all 15 residues of the telopeptide and the first 53 residues of the propeptide. Splicing probably occurs within the last glycine codon. The 112-bp HpaII fragment within the carboxy-terminal junction exon is present in both the gene clones and cDNA clones; its sequence was determined only from the cDNA clone<sup>31</sup>.

sequence for the presumptive propeptidase cleavage side: Asn-Phe-Ala-Ala $\downarrow$ Gln. As no homology can be found between the calf propeptide amino acid sequences located 5' to these in calf and the amino acid sequence which would result from the chick DNA sequence in this region, and as a CAG (the canonical splice junction sequence, see below) immediately precedes the codon for Asn, this exon presumably starts with the above conserved pentapeptide sequence. The following 14 residues, which include the first 3 residues in the triple-helical region, are indentical with the amino acid sequence derived from chick skin  $\alpha$ 2 collagen<sup>13</sup>. At the carboxy-terminal end<sup>8</sup>, the situation is similar except that larger portions of the propeptide and triple-helical coding regions are included in the exon coding for the telopeptide: a single exon codes for 15 residues of the helical region, all 15 residues of the telopeptide and the first 53 residues

of the propeptide. That these three structural domains (at both ends of the collagen molecule) are not separated by introns, as might have been expected, may possibly be explained in that they consist of a specific functional domain, namely the endopeptidase cleavage site.

### **Intervening sequences**

The 19 exons whose DNA sequences have been determined provide an equal number of intron-exon junction sequences (Table 1). In the triple-helical region, there is always an ambiguity as to the precise splice point due to the duplication of Gs at each end of the intron and there is a special problem in defining the exact splice points in the region which codes for the carboxy-terminal propeptide; three nucleotides at both junctions of exon

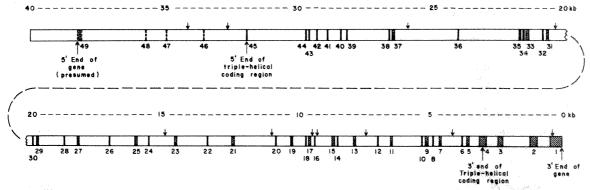


Fig. 6 Location of coding sequences in the pro α2 collagen gene. Forty kilobases of genomic DNA containing the pro α2 collagen gene are represented by two bars, both oriented 5' to 3' (left to right). Exons are indicated by vertical bars, numbered 1 to 49 (3' to 5'). The four exons at the 5' end of the gene have been identified only by electron microscope visualization of RNA-DNA hybrids by Ohkubo et al.6, and are thus indicated by dotted lines. The exon sizes in the helical region whose DNA sequences have not been determined have been indicated such that the small-large alternating pattern extends from exon 38 to exon 5; this is consistent with electron microscope measurements. Arrows (↓) indicate EcoRI sites. Exons whose DNA sequences have been determined are as follows. In this article and ref. 8: exon numbers 45 (amino-terminal structural domain junction); 44-41 (residues 4-72); 35-29 (residues 175-360); 16 (residues 676-693); 6 and 5 (residues 946-999); 4 (carboxy-terminal structural domain junction); 3, 2 and part of 1 (carboxy-terminal propeptide). By other researchers 2: 43-41 (residues 19-72); 27 and 26 (residues 379-429); 10 (residues 838-855); 9 (residues 856-891); 8 (residues 892-909). Only the ends of exon 9 have been sequenced, and therefore it has not been established whether this is one or two exons.

	Table 1 Intron-exon junction sequences						
	Residue						
	nos	Intron	Exon	Intron			
1	-3	TCATCTACAG	AATATG	GTAAGTATAT	(junction)		
2	4-18	TCTTCCCTAG	asn met GGTCCT gly pro	GTAAGTACAG	(triple-helical)		
3	19-36	TCTTGTCTAG	GGTACA gly thr	GTAAGTACAG			
4	37-54	TATTTGACAG	GGTGAT	GTAAGTCATT			
5	55-72	CATTTTTCAG	gly asp GGTCAA gly gln	GTAAGTATGT			
6	175-207	TCTCAAACAG	GGTCCC	GTGAGTACCT			
7	208-222	GCCTCCATAG	gly pro GGCGCT gly ala	GTAAGTATAG			
8	223-255	TTTGGAACAG	GGTGTT	GTAAGTGACT			
9	256-273	TGGACTCTAG	gly val GGTCCT	GTAAGTATGG			
10	274-309	GCTGAGGTAG	gly pro GGTAGA	GTAAGGTTTT			
11	310-327	TCACTTGCAG	gly arg GGCATG	GTAAGGCTTG			
12	328-360	TTGTTGTTAG	gly met GGAAGA	GTAAGTCTAG			
13	676-693	TCACACATAG	gly arg GGTCCT	GTAAGTCCTC			
14	946-963	TTCCCAATAG	gly pro GGCAGG	GTATGTGAAT			
15	964-999	TTTCTTCTAG	gly arg GGTGCT gly ala	GTGAGTATAA			
16	1,000-	AAAACTTTAG	GGCGCG	GTACGTGGTG	(junction)		
17		TTCCTCAAAG	gly g GGTCAG	ly GTATGTGATG	(carboxy-terminal propeptide)		
18		TCTTTTCAG	ly gln TTTTCT	GTAAGTAACA			
19		TCTCTTGCAG	phe ser AAA lys	ND			

ND, not determined.

17 are duplicated at the adjacent exon junction, so that four splice points are possible. The splice points in Table 1 have been aligned so that they conform with the GT---AG rule<sup>14</sup>; this requires splicing within the glycine codon at the ends of exons 16/17. With the sequences aligned in this manner, 11 introns begin with GTAAGT, and 7 others differ from this by a single base change. Eighteen introns end with (C/T)AG. These sequences agree well with those found for other eukaryotic genes and are indeed homologous to the 5' part of the U1 RNA which has been proposed to be involved in the splicing process<sup>15,16</sup>. Because every exon in the triple-helical region starts with a glycine residue, the first nucleotide in each is a G, as is usually found in other eukaryotic genes. However, the G which is commonly found at the 3' end of the exons in most genes occurs infrequently in the pro  $\alpha$ 2 collagen gene. There appears to be a strong bias against the use of G in the third position of codons in this gene.

The introns defined by DNA sequence analysis range in size from 86 bp to more than 2,000 bp. The average G+C content of the introns is about 35%, in striking contrast to the G+C-rich (62%) coding sequences, resulting in an average G+C content of 40%. The structure of the pro  $\alpha 2$  collagen gene therefore consists of many small G+C-rich coding regions embedded in A+T-rich stretches of DNA.

There seems to be little homology between different introns. Computer-generated homology plots 17 show that the longest homologies are  $\sim 10$  bp. However, some of the introns seem to contain DNA sequences which are repeated in other parts of the genome. EcoRI fragments G, H and I all hybridize to a large size range of DNA fragments on Southern blots of chicken DNA (J.W., unpublished data). The observation that some of the introns contain sequences which are repeated in distal parts of the genome is similar to that found for the chick X gene, which lies near the ovalbumin gene 18, and the conalbumin gene 19, but contrasts with the structure of the ovalbumin and globin genes, where the introns contain only unique DNA 26.21.

### Number of exons

The DNA sequence determinations of various regions of the chicken pro  $\alpha$ 2 collagen gene indicate that the structure of this gene is complex. By integrating the data from the various techniques used to characterize this gene, we deduce the detailed picture of the gene's structure presented in Fig. 6. The placement and sizes of the exons, indicated by vertical bars in the figure, have been determined by DNA sequence analysis, Southern blot restriction mapping and electron microscopy studies of genomic DNA hybridized to procollagen mRNA in R-loop conditions. The parts of pro  $\alpha^2$  collagen for which individual exons code are detailed in Fig. 6 legend. Certain details differ from the arrangement of exons presented by Yamada et al.9 and Ohkubo et al.6, deduced from electron microscopy studies and limited DNA sequence analysis. The DNA sequence analysis of multiple exons coding for contiguous parts of the protein ensure that the number of exons in these regions and their placement with respect to the map presented in Fig. 6 are correct.

We have indicated in Fig. 6 that the pro  $\alpha$ 2 collagen gene contains 49 exons. This is only an approximate number, as some of the exons whose DNA sequences have not been determined are identified only by electron microscopy, in which small exons or introns may be undetected. However, the figure is a fairly accurate representation of the location and minimal number of exons. Note that the density of exons in the 3' half of the gene is higher than that in the 5' half; there is more than twice as much coding information in the last 19 kbp of the gene as in the first 19 kbp. From the 14 triple-helical coding exons whose sequences were determined, the average exon size is ~70 bp. This predicts a total of 41 to 42 exons for the triple-helical coding region (excluding the junction exons). Adding to this the four exons coding for the carboxy-terminal propeptide and several for the amino-terminal propeptide, we predict a total of about 50 exons in the pro  $\alpha$ 2 collagen gene.

The highly interrupted nature of the pro  $\alpha$ 2 collagen gene requires the transcription of 38 kbp of DNA and the precise removal of about 50 introns to produce a single mature 5-kbp messenger RNA. The large number of splicing reactions which must take place certainly suggests that RNA splicing is in some sense processive, that is, that the enzyme would start at an intron/exon junction and search processively down the intron DNA for a complementary splice point<sup>22</sup>. An alternative random mechanism would probably result in the excision of one or more coding regions, producing an error frequency intolerable for a gene with 50 exons.

Regardless of whether the introns arose concurrently with the evolution of the gene or were inserted after the gene was developed, it seems clear that there must be a reason for maintenance for this highly interrupted structure. It is possible that the introns help to stabilize the coding sequences. Investigation of the silk fibroin gene, which also has highly internally repetitious coding sequences but only one intron in the entire gene, has shown that different alleles of the gene give rise to differently sized fibroin molecules<sup>23</sup>. The size differences appear to result from changes in the size of the repetitive parts of the genes, presumably a result of recombination between these parts in misaligned genes, that is, homologous unequal crossingover. By embedding the repetitive collagen coding sequences in nonhomologous DNA (the introns), the probability of homologous recombination would be reduced<sup>24</sup>, thus stabilizing the size and structure of the collagen molecule.

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#### Conclusion

The isolation of clones spanning 54 kbp of genomic DNA and DNA sequence determinations of over 4 kbp of this region, have shown that the chicken pro  $\alpha 2(I)$  collagen gene has a bizarre structure. The 5 kbp of coding sequences are spread out over about 38 kbp of genomic DNA, and are interrupted by some 50 introns. The exons coding for the amino- and carboxy-terminal ends of the triple-helical region also code for the telopeptides and part of the propeptides. The rapid accumulation of procollagen mRNA in embryonic connective tissue requires that the excision of the multiple noncoding regions in the primary transcript be both precise and efficient. Thus, we can now add a multistep assembly of coding regions in the nucleus to the multistep assembly of collagen chains (triple-helix formation, glycosylation, cleavage of propeptides, fibrillogenesis and crosslinking) in the cytoplasm and Golgi apparatus as a requirement for the production of collagen fibrils. One may wonder why the synthesis of this major component of the extracellular matrix has evolved into such an exquisitely complicated process.

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### The 26.13 MHz absorption line in the direction of Cassiopeia A

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It has recently been suggested that the absorption line  $\nu =$ 26.13 MHz that we had detected<sup>2</sup> in the direction of Cassiopeia A might be a recombination line due either to carbon (C631 $\alpha$ ) present in H I clouds or to heavier elements of hot gas. Observations reported here taken in February 1981 suggest that the low-frequency attenuation in the spectrum of Cas A is due to the presence of a cold cloud of ionized carbon.

Recombination lines can be defined by measuring adjacent lines, for example  $630\alpha$  and  $632\alpha$  in the present case. We carried out similar observations during the summer of 1978 although these were hampered by high interference. We have re-examined a series of the spectra near the line C630 $\alpha$  ( $\nu$  = 26.250 MHz) and averaged the maximum number of recordings not deteriorated by interference (total time 3 h—see Fig. 1). The resulting spectrum contains an absorption line whose frequency  $(\bar{\nu} = 26.254 \text{ MHz})$  agrees well with the calculation for the  $C630\alpha$  at the local standard of rest and with allowances made

for the line-of-sight velocities in the Perseus arm. The intensity is close to that of the line  $\nu = 26.13$  MHz. Neither the H630 $\alpha$ nor the  $H631\alpha$  line are observed. In February 1981 we performed more observations. Figure 2 shows the spectrum near the frequency 25.04 MHz, showing the line  $C640\alpha$  in absorption.

Thus, it seems reasonable to assume that we have observed a recombination line of carbon. However, more measurements are planned during the summer of 1981 (the most favourable time for observing Cas A) to check this assumption.

The decametric band spectral measurements were also carried out in other galactic directions, over a wide range of frequencies including both the <sup>14</sup>N lines and  $Hn\alpha$ ,  $Hen\alpha$  and  $Cn\alpha$  recombination lines which were not detected. The upper estimate of their intensities was  $\Delta T_{\rm L}/T_{\rm c} < 3 \times 10^{-4}$  (ref. 3).

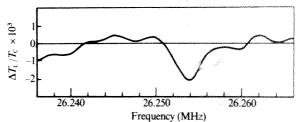


Fig. 1 The spectrum near the line C630a. The frequency corrected for motion of the Earth. Frequency resolution, 4 kHz; storage time, 3 h.

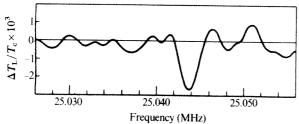


Fig. 2 The spectrum near the line  $C640\alpha$ . The frequency corrected for motion of the Earth. Frequency resolution, 2 kHz; storage time, 10 h.

If the observed absorption line were really due to ionized carbon, it would yield unexpected results.

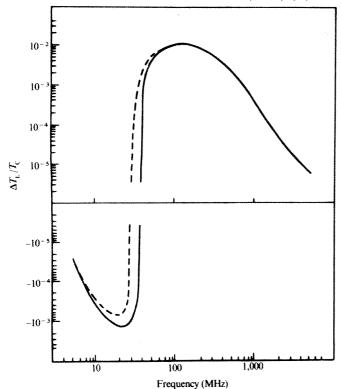
The frequency dependence of the carbon line intensity in the direction of Cas is interesting. Considering only frequencies  $\nu < 1,000 \, \mathrm{MHz}$  where the brightness temperature of Cas A is essentially higher than the electron temperature of the cloud, we have for the relative intensity of the recombination line4

$$\frac{\Delta T_{\rm L}}{T_{\rm c}} = -\tau_{\rm L}^* \left[ 1 - \frac{kT_{\rm e}}{h\nu} \left( 1 - \frac{B_n}{B_{n+1}} \right) \right] e^{-\tau_{\rm c}} B_n$$

where  $\tau_L^*$  is the equilibrium optical depth at the recombination line;  $\tau_c$  the optical depth in the continuum; and  $B_n$  a factor accounting for the deviation of the nth level from the local thermodynamic equilibrium conditions.

Figure 3 illustrates the relative intensity of carbon recombination lines as a function of frequency for  $N_e = 0.1 \text{ cm}^ T_e = 50 \text{ K}$  and measure of emission,  $ME = 0.07 \text{ cm}^{-6} \text{ pc}$ . The values of  $\Delta B_n/B_n$  for n > 500 are an extrapolation of earlier data<sup>5</sup>:  $\Delta B_n/B_n = 7 \times 10^{-6}$  at 25 MHz (solid line) and  $\Delta B_n/B_n = 10^{-6}$ 2×10<sup>-5</sup> at 25 MHz (dashed line).

Figure 3 shows that the lines observable in the decametre waveband are absorption lines; at 26 MHz the results which fit our measurements  $(\Delta T_{\rm L}/T_{\rm c} \sim 2 \times 10^{-3})$  are similar to those reported by Blake et al. Near 40 MHz the lines are extremely weak. They can be observed as emission lines at frequencies  $\sim$ 150 MHz, reaching quite high intensities (to  $10^{-2}$ ). This is also true for recombination lines of hydrogen with the same parameters as above. However, because the latter have never been detected either in the direction of Cas A (refs 3, 6, 7) or in



The calculated relative intensities of carbon recombination lines versus frequency

other directions, it suggests a low degree of ionization of the hydrogen clouds  $\zeta_H < 10^{-17} 1/c$ . The band of analysis used in the search for the  $H300\alpha$  (ref. 6) and  $H352\alpha$  lines (ref. 7) covered some carbon lines. The present calculations show the detectability of such lines for carbon clouds compared with the parameters given by Blake et al.1. In the spectrum of Shaver et al.<sup>7</sup>, several values have been excluded near the C352 $\alpha$  line ( $\nu = 150.214$  MHz), as allegedly deteriorated by interference. If the theoretical data given by Shaver<sup>4,5</sup> are correct, the carbon line should be observed in emission, with an intensity almost one order of magnitude higher than the detection threshold achieved. In this regard, observations in the frequency range 100-200 MHz would be of interest where emission recombination lines can be detected in cold clouds if they are sufficiently amplified by induced radiation.

Until recently, the decrease in the intensity of low-frequency radiation from Cas A was explained by the presence on the line of the sight of a H II region, with the electron concentration  $N_e \sim 1 \text{ cm}^{-3}$ , temperature  $T_e \sim 5,000 \text{ K}$  and  $ME \sim 100 \text{ pc cm}^{-3}$ (refs 7, 8). However, almost the same amount of free-free absorption can be caused in this direction by a cloud of carbon with  $N_e = 0.1 \text{ cm}^{-3}$ ,  $T_e = 50 \text{ K}$  and  $ME = 0.07 \text{ pc cm}^{-6}$ . Hence, the low-frequency attenuation in the spectrum of Cas A might well be due to the presence of a cold cloud of ionized carbon.

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### High-sensitivity detection of negative ions in the stratosphere

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Mass spectrometer composition measurements of stratospheric negative ions were first made by Arnold and Henschen<sup>1</sup> using a balloon-borne instrument. These initial measurements, which were taken at an altitude of 36.5 km, revealed that the major ion species are  $NO_3^-(HNO_3)_a$  and  $HSO_4^-(H_2SO_4)_b(HNO_3)_c$ . The suggestion that the unexpected HSO4 cores are formed from NO<sub>3</sub> cores by a reaction involving stratospheric sulphuric acid vapour was strongly supported by laboratory measurements of the relevant ion-molecule reactions by Viggiano and colleagues2. Meanwhile, major instrumental refinements in balloon-borne ion mass spectrometers, including increased mass range, sensitivity and mass resolution, have resulted in the detection of more massive HSO4(H2SO4), ions and in the extension of the height range from a thin layer around 35 km to a region extending from 23 to 39 km (refs 3, 4). Here we report in situ negative ion composition measurements of even higher sensitivity which led to the detection of previously unobserved ion species.

The instrument, which has been described in detail elsewhere<sup>3,5-7</sup>, consists of a cryogenically (liquid neon) pumped quadrupole mass filter with single ion detection by a channel electron multiplier operating in a pulse-saturated ion counting mode. The ions were drawn into the pumped chamber by applying 10 V to the front plate. The mass filter, which has a mass range of 0-276 AMU, can be operated in various modes differing in mass resolution and sensitivity. It also includes a so-called integral mode which allows the detection of ions having masses >276 AMU (see ref. 3). The ion detection limit of the present instrument is about 1 ion cm<sup>-3</sup>, which corresponds

to a fractional ion abundance of  $5 \times 10^{-4}$  at 34 km. This 20-fold improvement in sensitivity over previous instruments was obtained by a simplification of the ion optical system—an enhanced ion signal was obtained by removing ion lenses and focusing elements between the inlet orifice and the quadrupole. An additional increase in counting efficiency was achieved by placing a grid directly in front of and at equipotential with the detector channel multiplier.

The present measurements were taken on 26 September 1980 during a daytime flight over southwestern France. Figure 1 shows data obtained at the peak or 'float' altitude of 34 km at noon local time. The US Standard Atmosphere temperature for this location and time is 234 K (ref. 8). An additional measure of temperature was obtained by considering the proton hydrate positive ion spectrum taken during the same flight'. Taking the measured ratio of adjacent proton hydrates, H<sup>+</sup>(H<sub>2</sub>O)<sub>3</sub>/H<sup>+</sup>(H<sub>2</sub>O)<sub>4</sub>, a typical water mixing ratio of 4 p.p.m. and the laboratory thermodynamic data of Kebarle<sup>10</sup>, we calculate a temperature of 237 K at 34 km. As the US Standard Atmosphere temperature represents average stratospheric conditions and because our method for temperature determination has not been independently corroborated, we assume an average of 235 K with error bars of 5 K.

Two sample spectra are shown in Fig. 1. The spectrum of highest resolution or A-mode spectrum (Fig. 1a) and the expanded version (Fig. 1b) is the sum of 100 mass sweeps of 11 s each; the peak width at half height is  $\sim$ 4 AMU. The spectrum shown in Fig. 1c is the sum of 50 sweeps with the mass spectrometer in the B-mode; in this mode a larger ion sensitivity

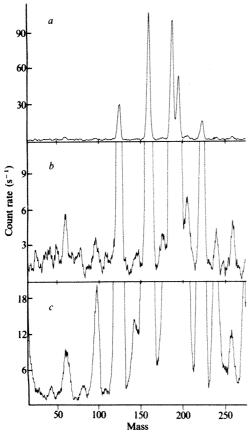


Fig. 1 Sample negative ion spectra measured at 34 km. The total scan time was 1,100 s and the dark count rate was 1 c.p.s. a shows major ions with the mass spectrometer set at its highest resolution (A-mode); b is the some A-mode spectrum expanded to show minor peaks; c was taken with mass spectrometer set for lower resolution but higher sensitivity (B-mode). The large apparent peak at the lower end of the B-mode scale is due to the integral signal at zero mass of the quadrupole<sup>3</sup>. Counting statistics were improved by subjecting raw data, divided into 1,000 channels along the mass scale, to a running mean averaging where the 'window' was smaller than the peak width.

Table 1 Mass numbers, ion identifications and fractional ion count rates of negative ions at 34 km

Mass (AMU)	Ion	Fractional ion count rate (%)
	Group I	
$125 \pm 0.5$	NO <sub>3</sub> ·HNO <sub>3</sub>	1.8
$160 \pm 0.5$	HSO <sub>4</sub> ·HNO <sub>3</sub>	6.5
$188 \pm 0.5$	$NO_3(HNO_3)_2$	6.2
$195 \pm 0.5$	HSO4·H2SO4	3.3
$223 \pm 0.5$	$HSO_4^{\sim}(HNO_3)_2$	0.9
	Group II	
$61 \pm 1$	NO <sub>3</sub> , CO <sub>3</sub>	0.1 - 0.4
$97 \pm 1$	HSO <sub>4</sub> , NO <sub>3</sub> ·HCl, CO <sub>3</sub> ·HCl	
$174 \pm 2$	NO <sub>3</sub> ·HNO <sub>3</sub> ·HOCl, HSO <sub>4</sub> ·HNO <sub>3</sub> ·H <sub>2</sub> O	
$206 \pm 2$	NO <sub>3</sub> (HNO <sub>3</sub> ) <sub>2</sub> ·H <sub>2</sub> O	
$240 \pm 2$	NO <sub>3</sub> (HNO <sub>3</sub> ) <sub>2</sub> ·HOCl, HSO <sub>4</sub> (HNO <sub>3</sub> ) <sub>2</sub> ·H <sub>2</sub> O	
$259 \pm 2$	HSO <sub>4</sub> ·H <sub>2</sub> SO <sub>4</sub> ·HNO <sub>3</sub>	
	Group III	<b>.</b>
$26 \pm 2$	CN <sup>-</sup>	0.05-0.1
$43 \pm 2$	CN-H <sub>2</sub> O	La constant de la con
$80 \pm 2$	NO <sub>3</sub> ·H <sub>2</sub> O, CO <sub>3</sub> ·H <sub>2</sub> O	1994
$109 \pm 2$	NO <sub>3</sub> ·HNO <sub>2</sub>	- Special Control of C
$133 \pm 2$	HSO <sub>4</sub> ·HCl	***
$142 \pm 2$	NO <sub>3</sub> ·HNO <sub>3</sub> ·H <sub>2</sub> O	te e e e e e e e e e e e e e e e e e e
$148 \pm 2$	HSO <sub>4</sub> ·HOCl	r.
$211 \pm 2$	HSO <sub>4</sub> ·HNO <sub>3</sub> ·HOCl, HSO <sub>4</sub> ·H <sub>2</sub> SO <sub>4</sub> ·H <sub>2</sub> O	and the same of th
$131 \pm 2$	HSO <sub>4</sub> ·H <sub>2</sub> SO <sub>4</sub> ·HCl	- Carlonnian
$248 \pm 2$	HSO <sub>4</sub> ·H <sub>2</sub> SO <sub>4</sub> ·HOCl	Section 1
$273 \pm 3$	HSO <sub>4</sub> (HNO <sub>3</sub> ) <sub>2</sub> ·HOCl,	data
	HSO <sub>4</sub> ·H <sub>2</sub> SO <sub>4</sub> ·HNO <sub>3</sub> ·H <sub>2</sub> O	4

Ions are separated into groups according to peak count rates. Adjustment in the fractional ion count rate was made for sulphuric acid clusters which are out of mass range of the instrument (see ref. 3).

is obtained at the expense of mass resolution (the peak width is  $\sim$ 7 AMU). The A-mode allows an unambiguous mass identification for the major peaks ( $\pm 0.5$  AMU). For the peaks near the detection limit the total signal is of the order of 100 ion counts per AMU; the dark current is about 20 counts per AMU interval (A-mode). Thus, poor counting statistics and associated poor peak shape result in a mass uncertainty of  $\pm 2$  AMU.

It can be seen that in the low mass region of Fig. 1 peak crowding and poor counting statistics hamper peak identification in the A-mode; in the B-mode, although the peak widths are larger, the minor peaks are more readily identified. In the high mass region, on the other hand, the A-mode spectrum is more informative. This is due to extended flanks of the dominant acid peaks in the B-mode spectrum which in many cases overlap and mask the much less intense minor peaks. Another feature evident in Fig. 1 is the change in abundance of some of the minor peaks between the A- and B-modes, notably the relative abundances of mass  $61\pm1$  to  $97\pm1$  AMU and mass  $240\pm2$  to  $259\pm2$  AMU. Although this difference may result partly from statistical fluctuations in the least sensitive A-mode, it could also be due to temporal and/or spatial variations in the ambient ion composition.

Table 1 is a compilation of observed mass peaks identified from spectra like those in Fig. 1 together with tentative ion assignments. Comparison of the integral mode and the resolving mode spectra reveals that most of the ambient ions have masses >276 AMU and thus could not be analysed. This result is consistent with other recent in situ observations<sup>3,4</sup>

To aid discussion, the ions listed in Table 1 have been separated into three groups according to ion signal strength. Group I consists of ions with fractional ion count rates >0.01 and whose mass numbers can be identified to within  $\pm 0.5$  AMU. These ions belong to the families  $NO_3^-(HNO_3)_a$  and  $HSO_4^-(H_2SO_4)_b(HNO_3)_c$  and have all been observed previously with fractional ion count rates similar to those reported here  $^{1.3.4}$ . The minor ions fall into two groups: those with fractional ion count rates between 0.001 and 0.004 are placed in group II and

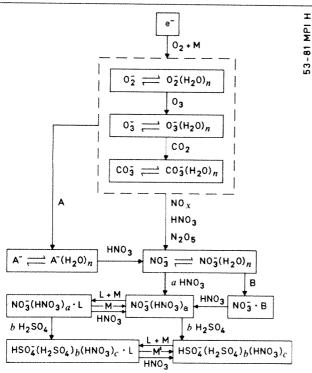


Fig. 2 Stratospheric negative ion reaction scheme including proposed minor ion species. L refers to the ligands H<sub>2</sub>O, HCl and HOCl. Hydrate equilibria are established quickly due to the relatively high water concentrations.

those with count rates between 0.005 and 0.001 in group III. In general, the peaks in group III are the most poorly defined and contain the largest uncertainty; nevertheless, their count rates are significantly above the background signal. The relatively higher count rates and better peak shapes of group II ions allow a mass number determination of  $\pm 1\,\mathrm{AMU}$  compared with  $\pm 2\,\mathrm{AMU}$  for the group III ions. In some cases, such as with  $174\pm 2\,\mathrm{AMU}$ , the mass position cannot be accurately determined because of the presence of an adjacent major peak.

As with the major negative ions, the minor ions seem to contain  $NO_3^-$  and  $HSO_4^-$  cores. There are, however, at least two cases where this does not apply; the core ion for the masses 26 and 43 is tentatively identified as  $CN^-$ . Also, due to the limited accuracy in mass number identification, we cannot exclude a contribution from  $CO_3^-$  and  $CO_3^ CO_3^-$  H<sub>2</sub>O to the peaks at  $CO_3^-$  and  $CO_3^-$  H<sub>2</sub>O to the peaks at  $CO_3^-$  and  $CO_3^-$  other than HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>. These ligands are tentatively identified as H<sub>2</sub>O, HCl, HNO<sub>2</sub> and HOCl. Although certainly not unique, these identifications seem reasonable; such Brönsted-Lowry acids are known to bond strongly to negative ions 11. H<sub>2</sub>O, the weakest of the acids, is also a likely ligand due to its large abundance. Arijs et al. 4 have also recently reported several minor ions containing H<sub>2</sub>O ligands.

As a certain ambiguity exists in the assignment of cluster ions on the basis of mass alone, we have also used physicochemical arguments to obtain ion identifications. We expect water as a second or third ligand, for example, only if the precursor cluster ion has a relatively high abundance. This is due to the relatively weak  $H_2O$  ligand bonds and to the general decrease in free energy of bonding with increasing ligand number 12. Thus, the ion at  $142 \pm 2$  AMU could well be  $NO_3^-HNO_3^-H_2O$  or the ion at  $206 \pm 2$  AMU  $NO_3^-(HNO_3)_2 \cdot H_2O$ ; the precursor clusters in these cases,  $NO_3^-\cdot NHO_3$  and  $NO_3^-(HNO_3)_2$ , are major ions in the spectrum. On the other hand, the ion at 248 AMU is not given the structure  $HSO_4^-\cdot H_2SO_4\cdot HCl\cdot H_2O$  because of the low abundance of the  $HSO_4^-\cdot H_2SO_4\cdot HCl$  ion. The masses which cannot be decided on this basis are given in Table 1 with multiple cluster ion assignments.

The core ion CN should react with HNO3 via

$$CN^{-} + HNO_3 \rightarrow NO_3^{-} + HCN \tag{1}$$

as the electron affinity of CN  $(3.8\,\mathrm{eV})^{13}$  is smaller than that of NO<sub>3</sub>  $(4.0\,\mathrm{eV})^{14}$  and the bond energy of HCN exceeds that of HNO<sub>3</sub> (ref. 15). The absence of ions containing CN<sup>-</sup> cores with HNO<sub>3</sub> ligands also suggests that HNO<sub>3</sub> reacts with the core rather than attaches to it.

Proposed reaction schemes for stratospheric negative ions include the core ion CO<sub>3</sub>, but do not take CN<sup>-</sup> reactions into account<sup>16</sup>. Due to the large electron affinity of CN, the CN<sup>-</sup> core may be formed from O<sub>2</sub>, O<sub>3</sub> or CO<sub>3</sub> cores by reactions involving a CN compound, probably HCN. By assuming that CN<sup>-</sup> cores are lost primarily by a reaction involving HNO<sub>3</sub> and that their abundances reach a kinetic steady state, we estimate the HCN abundances as being of the same order as the HNO<sub>3</sub> abundance. This qualitative result is consistent with recent IR absorption measurements of Coffey et al.<sup>17</sup>.

Ions containing the ligands H<sub>2</sub>O, HCl, HNO<sub>2</sub> and HOCl would be expected to be lost by HNO<sub>3</sub> switching reactions because, for the cluster ion sizes considered, a ligand bonds more strongly the higher its gas phase acidity <sup>10</sup>. As the gas phase acidity of HNO<sub>3</sub> exceeds those of H<sub>2</sub>O, HCl, HNO<sub>2</sub> and HOCl (refs 15, 18), nitric acid should exoergically displace these ligands. It is also conceivable that ions containing such weakly bound ligands as H<sub>2</sub>O are lost primarily by thermal dissociation (see Fig. 1).

Fragmentation during ion sampling is known to be minimal for the strongly bound clusters with sulphuric and nitric acid ligands<sup>6,7</sup>; but an important question is whether the weakly bound H<sub>2</sub>O ligands are affected by fragmentation. This question might be answered by considering the hydration equilibria of the observed ions. However, for a hydration equilibrium to obtain. as opposed to a dynamic steady state, the dominant loss process for a hydrate ion A-H<sub>2</sub>O must be thermal dissociation and not switching with an ambient species of higher gas phase acidity. Using the forward rate constants and association free energies for several negative ion hydrations given by Fehsenfeld and Ferguson<sup>19</sup>, we calculate thermal dissociation rates which are typically an order of magnitude faster than nitric acid switching reactions calculated with an upper limit gas collision rate constant. Thus, if the hydrate ions are not significantly fragmented during ion sampling, the measured fractional abundances for A-H<sub>2</sub>O and A ions should reflect ion hydration equilibria of the form

$$\frac{[A^- \cdot H_2O]}{[A^-][H_2O]} = k_{eq} = \exp(-\Delta G/RT)$$

From the measured fractional ion count rates and a typical 4 p.p.m.v. water mixing ratio  $^{20}$ , we calculate the free energy differences,  $\Delta G$ , shown in Table 2. These values are compared with laboratory  $\Delta G$ s for the hydrations of  $CN^-$  and  $NO_3^-$ ; in the absence of data for the hydration of  $NO_3^-$ ·HNO $_3^-$  and  $NO_3^-$ (HNO $_3$ )<sub>2</sub>, we use available data for the hydration of  $NO_3^-$ ·H $_2O$  and  $NO_3^-$ (H $_2O$ )<sub>2</sub>. In view of the uncertainty in atmospheric parameters such as temperature and water concentration, the good agreement shown in Table 2 is fortuitous and no rigorous conclusions can be drawn from it; nevertheless, we feel that it provides further support for our ion

**Table 2** Free energies of hydration derived from observed fractional ion count rates and water number density of  $8\times10^{11}$  cm<sup>-3</sup>

	$-\Delta G$ (kcal mol <sup>-1</sup> )				
A	Association order	Derived from data	Literature		
CN <sup>-</sup>	1	$8.3 \pm 1$	9.1*		
$NO_3^-$	1	$8.0 \pm 1$	7.9*, 8.7†		
NO <sub>3</sub> ·HNO <sub>3</sub>	2	$6.7 \pm 1$	7.3†‡		
$NO_3(HNO_3)_2$	3	$6.7 \pm 1$	6.0†‡		
HSO <sub>4</sub> ·H <sub>2</sub> SO <sub>4</sub>	2	$6.4 \pm 1$	and an entire		

<sup>\*</sup> Ref. 12.

<sup>†</sup> Ref. 28.

 $<sup>\</sup>ddagger \Delta G$  for the addition of H<sub>2</sub>O to NO $_3^{\circ}(\text{H}_2\text{O})_n$  of the same association order.

identifications as well as for the contention that the hydrates are not severely fragmented during ion sampling.

Ions containing the acid ligands HCl, HNO2 and HOCl may be formed either by three-body association or by switching of the corresponding ligand molecules. If association occurred at a gas collision rate and if HNO, switching was indeed the major sink for these ions, a steady-state treatment would yield an estimate for the abundances of HCl and HOCl of 0.1 to 1 times that of the HNO<sub>3</sub> concentration and for HNO<sub>2</sub> as roughly equal to HNO.

Although stratospheric IR absorption measurements show the HCl abundance to be comparable with that of HNO3 at 34 km (ref. 21), HOCl has not been observed. Calculations based on laboratory rate data of HOCl reactions and photolysis predict HOCl abundances which may be as large as that of HNO<sub>3</sub> (refs 22-24 and P. Crutzen, personal communication). We feel, therefore, that the identification of HCl and HOCl ligands attached to stratospheric negative ions is at least plausible.

Nitrous acid is another trace gas which has not been observed in the stratosphere. An important source of this species is the reaction NO+OH+M→HNO<sub>2</sub>+M and the main loss path is photolysis<sup>25</sup>. Photolysis of HNO<sub>2</sub>, however, is much more efficient than that of HNO<sub>3</sub> so that one would not expect the abundance of HNO2 to be as high as is apparent from the present measurements (P. Crutzen, personal communication). Clearly, further atmospheric as well as model investigations of this species are needed.

Finally, we note that HNO<sub>3</sub> concentrations may be derived from the present measurements by a method which relies on the existence of a kinetic steady state with respect to the nitric and sulphuric acid cluster ions taken as a whole. The common precursor of these clusters is the NO<sub>3</sub> hydrate family—seen here for the first time—and the common sink is ion—ion recombination. The steady-state continuity equation is simply solved for the HNO<sub>3</sub> concentration; it is

 $[NO_3^-(H_2O)_a]k[HNO_3] = [NO_3^-(HNO_3)_a$ 

 $+HSO_4^-(H_2SO_4)_b(HNO_3)_a]\alpha n_+$ 

where k represents the rate constants for HNO3 association and switching with  $NO_3^-(H_2O)_{\infty}$   $\alpha$  is the ion-ion recombination coefficient, and  $n_+$  is the total positive ion concentration. Taking  $k = 10^{-9}$  cm<sup>3</sup> s<sup>-1</sup> (Fehsenfeld *et al.*<sup>14</sup> report a lower limit of  $5 \times 10^{-10}$  cm<sup>3</sup> s<sup>-1</sup>),  $\alpha = 10^{-7}$  cm<sup>3</sup> s<sup>-1</sup> (refs 6, 7) and  $n_+ = 2,000$  cm<sup>-3</sup>, we obtain a HNO<sub>3</sub> concentration of  $7 \times 10^7$  cm<sup>-3</sup>. If, alternatively, the HNO<sub>3</sub> concentration is derived from the abundance ratio of NO<sub>3</sub>(HNO<sub>3</sub>)<sub>2</sub> and NO<sub>3</sub>·HNO<sub>3</sub> (equilibrium method, see ref. 26), one obtains values which depend strongly on the set of thermodynamic data used. The data of Davidson et  $al.^{27}$  ( $\Delta H = -18.3$  kcal mol<sup>-1</sup> and  $\Delta S = -22.1$  entropy unit) give  $7 \times 10^7$  cm<sup>-3</sup>, the data of Lee *et al.*<sup>27</sup> ( $\Delta H = -17.7$  kcal mol<sup>-1</sup>,  $\Delta S$ not measured, we calculate -20.1 e.u. from  $\Delta G$  values of Davidson et al.) give  $9.6 \times 10^7$  cm<sup>-3</sup>, and those of Wlodek et al.<sup>29</sup>  $(\Delta H = -16.3 \text{ kcal mol}^{-1}, \Delta S = -23.1 \text{ e.u.})$  give  $9 \times 10^9 \text{ cm}^{-3}$ . Comparison of the above inferred HNO<sub>3</sub> concentrations reveals agreement between the steady-state and equilibrium methods if the thermodynamic data of Davidson et al. are used. These values are also in reasonable agreement with recent model calculations of P. Crutzen (personal communication) which give an HNO<sub>3</sub> concentration of  $7 \times 10^8$  cm<sup>-3</sup> at 34 km. A word of caution is in order, however, concerning the degree of agreement in the above derived HNO3 concentrations. Considering the uncertainties in input parameters such as temperature and kinetic and thermodynamic data, this close agreement is certainly fortultous; it does, however, provide grounds for optimism about the use of ion measurements to deduce stratospheric nitric acid concentrations. Laboratory kinetic and thermodynamic studies of the relevant ions as well as improved in situ measurements are needed to improve our understanding of minor negative ions. Planned atmospheric measurements are aimed at an extended height range and an unambiguous mass identification of the minor ions.

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### Near-edge X-ray absorption spectra for metallic Cu and Mn

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The measurement of X-ray absorption fine structure of metalsboth in the extended region (EXAFS) as well as in the near edge region (XANES)—has been widely discussed (see refs 1-6 for Cu and refs 7-9 for Mn). The recent availability of luteuse X-ray fluxes from storage rings has usually been exploited for EXAFS leaving the XANES often with poorer resolution than earlier work performed on conventional sources (for example, compare the near edge structure for copper in ref. 1 with refs 3 or 6). In addition, whilst the theory and analysis of EXAFS is relatively well-established. 1,18, a theory for the strong scattering regime near to the absorption edge has only recently been developed<sup>13</sup>. We report here the first high resolution XANES spectra for Ca and Mn which were performed at the SRS storage ring at Daresbury. Although both metals have close-packed structures consisting of atoms of similar size their local atomic structure is different in detail. Significant differences are found in their respective XANES reflecting the sensitivity of this region of the X-ray absorption fine structure to the local atomic structure. Spectra for the two metals have been analysed using the new multiple scattering formalism. This is a real space calculation and unlike a conventional band structure approach it does not require structural periodicity but works from the local arrangement of atoms.

K-edge absorption spectra for f.c.c. Cu (8.98 keV) and  $\alpha$ -Mn (6.54 keV) are presented in Fig. 1a, b with the respective

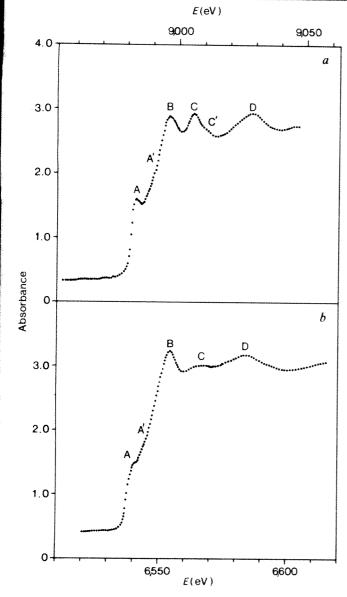
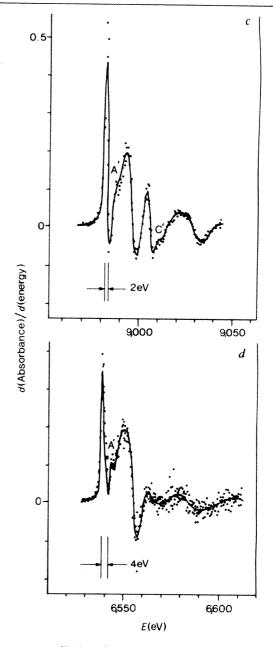


Fig. 1 XANES spectra for f.c.c. Cu (a) and α-Mn (b). Positions of peaks A, B, C and D are given in Table 1. Derivative XANES spectra for f.c.c. Cu (c) and α-Mn (d). Positions of A' and C' are given in Table 1. A cubic spline is included to guide the eye.

derivative spectra in Fig. 1c, d. The raw unsmoothed data are presented demonstrating the excellent signal-to-noise ratio obtained. Measurements were made at room temperature in transmission using 10-µm metallic foils supplied by Goodfellow Metals. The Cu foil was 99.99% pure and the Mn foil 98.7% pure (encapsulated in polyethylene). A Si 220 channel cut crystal diffracting vertically was used to monochromatize the white beam. Argon-filled ion chambers were used for detection in the conventional arrangement<sup>12</sup>. The 1-mm high entrance slits at 16 m from the source subtended an angle  $\Delta\theta$  of 13 arc s. This is two or three times larger than the angle subtended by the source or the width of the crystal rocking curve at these wavelengths and therefore defines the instrumental resolution. For the Si 220 crystal this is given by  $5.7 \times 10^{-5} \tan \theta \ \Delta \theta$  which for the Cu and Mn edges equals 1.5 and 0.8 eV respectively. ( $\theta$  is the Bragg angle given by  $\lambda = 3.84 \sin \theta$  Å). The 2-eV wide peak on the leading edge of the Cu spectrum (Fig. 1a) demonstrates the resolution achieved. The instrumental resolution was chosen to match the inherent spectral broadening. This derives from the finite lifetimes of the photoemitted electron and the core hole. Of these the core hole lifetime makes the principal contribution when the energy of the photoelectron is low. For Mn and Cu the inverse hole lifetime broadening is between 1 and 2 eV.



The spectra displayed in Fig. 1 were obtained during the commissioning period of the SRS when the machine was running with 10 mA of electron beam at an energy of 1.5 GeV. The comparatively low energy meant harmonic spectral contamination was minimized. However, coupled with the low beam current and the narrow entrance slits (1×10 mm), this reduced the photon flux at the sample  $^{13}$  to  $\sim 5 \times 10^7$  photons s<sup>-1</sup>. Data collection times resulted in typically 108 photons per point giving an integrated photon limited signal-to-noise ratio of 104. This value which compares well with the point-to-point noise recorded in the featureless parts of the spectra and indicates that there was no significant contribution from the detection system or from the residual scattered radiation in the vicinity of the ion chambers. For the SRS running at 2 GeV and 200 mA an improvement of at least an order of magnitude in signal-to-noise ratio is anticipated.

There are four strong peaks in the first 60 eV of fine structure for f.c.c. Cu. These are marked A, B, C and D in Fig. 1a, b. There is also some finer structure evident in the derivative spectrum (for example, A' and C' in Fig. 1c, d). The relative peak positions are listed in Table 1. Similar features also occur in the  $\alpha$ -Mn spectrum but the amplitudes are weaker on the whole (Fig. 1a, b) and the precise positions (Table 1) are different. This becomes clear by comparing the derivative spectra (Fig. 1c, d) where the similarity between f.c.c. Cu and  $\alpha$ -Mn only holds over the first

20 eV. The atomic volumes of copper and manganese are 11.7 and 12.1 Å<sup>3</sup> respectively and both have close-packed structures<sup>14</sup>. The differences in XANES almost certainly stem from the differences in local structure as other authors have concluded<sup>15</sup>. We will return to this point later.

Basically most fine structure in X-ray absorption spectra results from the scattering of the photo-emitted electron between the absorbing atom and those atoms surrounded it. The EXAFS at high energies (>50 eV above the absorption edge) is well described by single scattering theory10. In the XANES region close to the edge multiple scattering of the photoelectron plays an increasingly important part. As a result XANES in principle is sensitive to higher order atomic correlations<sup>11</sup> in addition to the pairwise correlations obtainable from EXAFS (or from X-ray or neutron diffraction, all of which are weak scattering probes). This means the fine structure is not simply interpretable as a combination of Fourier components—one for each shell of atoms9. Recently, Durham et al.11 extended the single scattering treatment of EXAFS4 to incorporate multiple scattering. In this approach the local density of states (the local electronic band structure 16,17) is built up by considering explicitly the relevant electron scattering paths within a finite portion of the system. Moreover in our theory the X-ray absorption cross-section is calculated in a real space representation, thereby removing the requirement, in band structure calculations, for relatively simple periodic structures.

Multiple scattering theory for XANES has been used successfully to predict the K-edge structure of f.c.c. Ag

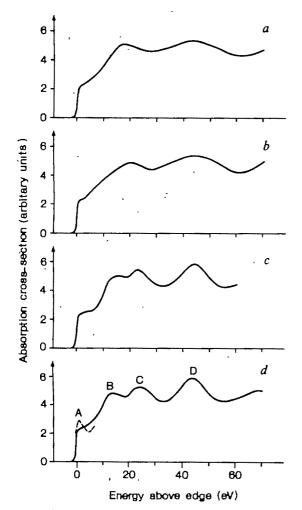


Fig. 2 Multiple scattering calculations for different cluster sizes of f.c.c. Cu; a, 1 shell; b, 2 shells; c, 3 shells; d, 4 shells. These consists of 12 atoms at 2.55 Å (1st shell), 6 atoms at 3.61 Å (2nd shell), 24 atoms at 4.42 Å (3rd shell) and 12 atoms at 5.09 Å (4th shell). The broken line in curve d represents the results of removing the broadening altogether in the vicinity of the edge.

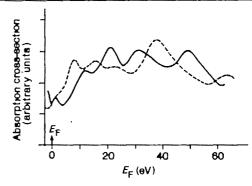


Fig. 3 Multiple scattering calculations for Mn in hypothetical b.c.c. (solid curve) and f.c.c. (dashed curve) structures, each with the same atomic volume as  $\alpha$ -Mn. Four shells of neighbour atoms are included in both calculations, and the Fermi level,  $E_{\rm F}$ , is an estimate consistent with band structure calculations  $^{20}$ .

(25.5 keV)11. The near edge structure of f.c.c. Cu shown in Fig. 1 is qualitatively similar to, but rather more pronounced than, that of Ag, and accordingly provides a more decisive test of the theory. Figure 2 shows a sequence of calculations for f.c.c. clusters starting with one shell and running up to four shells. Clearly the final calculation for a 55-atom cluster gives quite good agreement with experiment (Fig. 1). The four main peaks A, B, C and D are all predicted and their positions are accurate practically to within the experimental spectral resolution of  $\pm 1.5$  eV (Table 1). The relative amplitudes of the peaks as well as their individual asymmetries are also reproduced. Compared with experiment, however, the amplitude of the theoretical fine structure oscillations is approximately double what it should be. Whilst the calculated spectra have been broadened by 2.7 eV to match the inverse hole lifetime, no allowance has been made for the energy dependent inverse electron lifetimes, shakeup/shake-off effects or thermal disorder-all of which will decrease the amplitude of the fine structure oscillations. Our results are in good agreement with the APW band structure calculations of Wakoh and Kubo<sup>15</sup> (see Table 1). The rather similar K-edge spectrum of Ni has been investigated by Szmulowicz and Pease 18 using an APW calculation) who also discuss the origin of the main low-energy structures.

The XANES calculations for different sizes of cluster shown in Fig. 2 emphasize the sensitivity of this region to many shells of atoms. For instance, the four peaks of f.c.c. Cu are only reproduced when at least the third shell of 24 atoms at 4.42 Å is included. EXAFS is also sensitive to these outer shells, but this region of the absorption fine structure is dominated by the scattering effects of the first shell of 12 atoms at 2.55 Å (ref. 19).

The allotropes of Mn have extremely distorted structures of which  $\alpha$ -Mn is the usual form<sup>14</sup>. The overall packing density in  $\alpha$ -Mn is close to that in the b.c.c. structure but the local structure

Table 1 Peaks measured in the near edge structure of f.c.c. Cu and  $\alpha$ -Mn\* and the calculated positions for f.c.c. Cu using multiple scattering theory† with the APW band structure values‡

	A (eV)	A' (cV)	B (eV)	C (eV)	C' (eV)	D (eV)
f.c.c. Cu Experiment	2.(4)	5.(1)	14.(0)	24.(8)	28.(3)	45.(6)
Theory (present work) Theory	1.(5)		14.(6)	24.(4)		44.(2)
(ref. 15) α-Mn	2.(8)		14.(3)	23.(2)		
Experiment	3.(6)	5.(7)	17.(0)	28.(2)		45 (8)

All peak positions are taken relative to the first maxima in the derivative spectra. The literature values for the first maxima for Cu and Mn are 8,980.3 and 6,538 0 eV respectively <sup>21</sup>.

<sup>\*</sup> From Fig. 1a, b.

<sup>†</sup> From ref. 11 and Fig. 2.

<sup>‡</sup> From Fig. 1 of ref. 15.

shows few similarities. In particular there are four distinct first coordination shells containing between 12 and 16 atoms. The calculated XANES for Mn in hypothetical b.c.c. and f.c.c. structures with the same atomic volume as  $\alpha$ -Mn is shown in Fig. 3. The obvious differences between the two calculations demonstrates their sensitivity to local structure. On the other hand the f.c.c. Mn calculation shows clear similarities with the f.c.c. Cu calculations, but contains slightly more structure. In the same way the b.c.c. Mn calculation is qualitatively similar to the calculations of Wakoh and Kubo<sup>15</sup> on b.c.c. V and Fe. Comparison with experiment in Fig. 1 shows, however, that neither the b.c.c. nor f.c.c. Mn calculations agree well with the spectrum of  $\alpha$ -Mn. Although there are experimental similarities

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between a-Mn and Cu XANES, the amplitude of the oscillations are smaller for  $\alpha$ -Mn; for instance, peak A in the Cu spectrum becomes a shoulder for Mn. Evidently the quasidisordered nature of the local structure in  $\alpha$ -Mn tends to wash out the sharp features of a more symmetrical structure. Preliminary multiple scattering calculations for the first shell of  $\alpha$ -Mn display a significant variation in the fine structure for different inequivalent sites, the amplitude of which diminishes when an average is taken. More detailed calculations will be published later.

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### Solid-state reduction of iron in olivine -planetary and meteoritic evolution

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Iron-nickel metallic particles have been reported in meteorites<sup>1</sup> and lunar<sup>2-5</sup> and terrestrial<sup>6,7</sup> rocks. The origin of these metallic particles is not unique as they may be formed by (1) condensation from a primordial solar nebula<sup>8</sup>; (2) crystallization from a melt; and (3) subsolidus reduction reactions under low oxygen or sulphur fugacity. We report here an electron microscopy study of the solid-state microstructural development in olivine single crystals (Fo<sub>92</sub>) in which half of the iron has been reduced to the metallic state by a gas-solid interaction in the temperature range 950–1,500 °C. The reaction,  $Fo_{92} \rightarrow Fo_{96} + metallic Fe(Ni$ in solid solution) + pyroxene, begins with a homogeneous transformation involving fine-scale metallic precipitates resembling Guinier-Preston zones'. The microstructure develops by the growth of the first-formed precipitates during an Ostwald ripening process9 in which the precipitates located in the dislocation sub-boundaries develop in preference to precipitates in the subgrains. On the other hand, pyroxene is first observed to nucleate heterogeneously at pre-existing dislocations and its coarsening rate is more than an order-of-magnitude faster than that of the metallic phase. Besides the textural similarity of the observed microstructures with that reported for some of the lunar materials2, these results have important implications for the physical models of accretion of terrestrial planets, planetesimals and meteorites<sup>10</sup>, especially with respect to the distribution of siderophile elements. The rate of reaction observed here places constraints on models for the formation of the Earth's core by segregation of a metallic phase with or without reduction.

Single crystals of San Carlos olivine, Fo92, were heated under controlled oxygen fugacity,  $f_{O_2}$ , to temperatures in the range 950-1,500 °C and the conductivity of the samples measured These results indicated that the time-dependent variation in electrical conductivity was associated with a change in the

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oxidation state of iron. One sample (B) that had experienced reducing conditions during conductivity measurements was selected for electron microscopy studies. In addition, sample A was heated in the experimental assembly without conductivity measurements to study the early stages of the reduction reaction—it spent 1 h at 1,400 °C, 3 h at 1,300 °C and 1 h at 1,200 °C in CO/CO2 mixtures defined by line A in Fig. 1. Sample B was at 1,500 °C for 1.5 h, 1,400 °C for 2 h and 1,300 °C for 21 h in H<sub>2</sub>/CO<sub>2</sub> mixtures defined by region B (Fig. 1). The conductivity of sample B decreased by 50% in the first 2 h but remained unaltered during the next 23 h.

Assuming that the conductivity decrease is due to Fe loss from the olivine 11, then the reaction of olivine with the imposed  $f_{O_2}$  is completed within the first 2 h at these temperatures. Consequently, sample A should represent the earliest stages in the nucleation and growth of the metallic phase produced by the reduction reaction, while sample B would indicate the microstructural development in the coarsening process. To determine the growth rate parameters, all kinetic data were standardized to 1,300 °C. After the reaction, the composition of the matrix in sample B was Fo<sub>96</sub>, while its initial nickel content of 0.33 wt% was reduced to a level that was undetectable. Because of the homogeneous distribution of the fine-scaled precipitates in sample A, it was not possible to determine the matrix composition.

The microstructure of sample A was variable. The first indications of the reduction process were the matrix strain-field contrast effects<sup>12</sup> shown in Fig. 2a. These effects were homogeneously distributed in this region of the sample and not markedly affected by the pre-existing dislocations. However, in other areas, the dislocations had precipitates attached to them (Fig. 2b). Using the energy dispersive system attached to the JEOL 200 C microscope, the precipitate was analysed as a pyroxene. Unfortunately no extra diffraction information was found in the fine beam (0.05 µm) microdiffraction pattern. Sample B contained 0.2-1.5 µm blebs of iron-nickel (2 wt% Ni) distributed predominantly in dislocation sub-boundaries. These precipitates (M), kamacite, (identified by selected area electron diffraction), were located in the edge-dislocation sub-boundaries composed of b = [100] and b = [001] dislocations but were absent from cross-arrays of screw dislocations (Fig. 3a). Scanning electron microscopy indicated that the coarsening process for the metallic phase occurred throughout the bulk of the sample (Fig. 3b) and high-voltage microscopy (0.9 MeV) confirmed the distribution of the phase in thick sections (4-

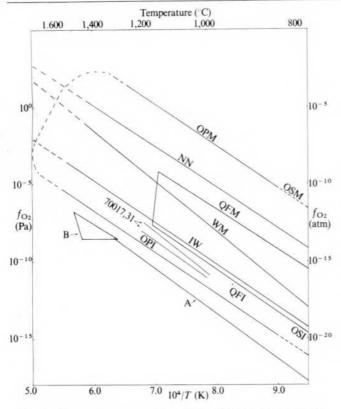
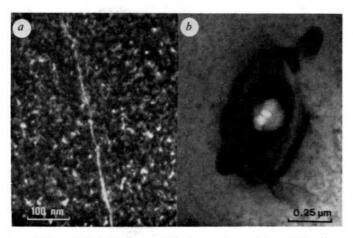


Fig. 1 Temperature versus  $f_{\rm O2}$  stability of olivine (after ref. 37) with 10% fayalite and pure fayalite, respectively compared with various oxygen buffers and lunar samples. OPM (olivine, pyroxene, magnetite) and OSM (olivine, silica, magnetite) delineate the oxidation of olivine with 10% fayalite. Reduction of olivine of that composition is indicated by OPI (olivine, pyroxene, iron) and OSI (olivine, silica, iron). For fayalite, the oxidation boundary is indicated by QFM (quartz, fayalite, magnetite) and QFI (quartz, fayalite, iron) (see ref. 34 for detailed discussion of these stability fields). The buffers NN (nickel-nickel oxide), WM (wisite-magnetite), and IW (iron-wüstite) are shown for comparison. Also shown is the intrinsic  $f_{\rm O2}$  determined for lunar basalt  $^{35}$  and the experimental paths A and B discussed in the text.

 $5 \mu m$ ). However, the pyroxene that formed during the reduction reaction was more heterogeneously distributed than in sample A, and, when observed, had grown to precipitates larger than  $5 \mu m$ . It had a high density of planar faults lying in the (100) planes that produced streaks in the diffraction pattern; there were also clinoenstatite lamellae up to  $0.1 \mu m$  wide in the orthopyroxene<sup>13</sup>. Although the starting material had a dis-



**Fig. 2** a, A dark-field image of the fine-scale precipitates of Fe-Ni in the olivine matrix—the dislocation has not acted as a preferred nucleation site: sample A. b, A pyroxene precipitate located at a dislocation loop: sample A.

location density of  $4(\pm 2) \times 10^5$  cm<sup>-2</sup> with widely spaced (100) and (001) dislocation sub-boundaries ranging from 50 to 100  $\mu$ m, sample B had an average subgrain size of 2.5  $\mu$ m and a free dislocation density of  $6(\pm 3) \times 10^8$  cm<sup>-2</sup>.

Figure 4 illustrates what is postulated to be the coarsening process for the metallic phase. The precipitate  $(M_1)$  within the subgrain is dissolving while the precipitate  $(M_2)$  at the subboundary coarsens. The dissolution reaction at  $M_1$  involves predominantly lattice diffusion with some minor pipe diffusion along the attached dislocation while the growth process of  $M_2$  occurs by pipe diffusion down the dislocation in the sub-boundary. This is the most likely mechanism for the growth of the few larger iron-nickel particles observed in the SEM (Fig. 3b).

It is apparent that the nucleation and growth processes of the metallic and silicate phases are different. The earliest evidence of the pyroxene is at pre-existing dislocations while the matrix still shows the strain-contrast effects, assumed to be the iron-nickel pre-precipitates, similar to Guinier-Preston (GP) zones<sup>9</sup>. However, incoherency at the interphase boundaries soon develops <sup>14,15</sup> producing both interfacial and lattice dislocations.

The growth rate of the pyroxene is difficult to estimate but the coarsening rate of the metallic phase can be calculated from the conductivity measurements on sample B, using a diffusion-controlled model for precipitate growth 16 and Fe diffusivity in olivine 17. The effects of pressure are important for reactions that may occur in the mantle. Pressure influences the diffusivity of Fe through the PV term in the general diffusion equation 18, where V is the activation volume for the rate-controlling step. One experimentally-determined value of V in olivine, based on the dislocation recovery rate, is 11 cm3 (ref. 19). This dislocation climb-related process is probably controlled by oxygen diffusivity. In another study of the rheological behaviour of olivine in the upper mantle, a value of 7 cm3 was used, assuming that both power-law creep and diffusional flow were controlled by oxygen ion diffusivity  $^{18}$ . There are no reported values of Vfor Fe diffusion in olivine, so a range of values-0, 1, 7 and 11 cm3-has been used to calculate precipitate sizes. Pressure and temperature influence the stability of olivine. To depths of 400 km (150 kbar), olivine is stable but with increasing depth, it transforms according to the following<sup>20</sup>: at 400 km olivine transforms to the β-polymorph; in the range 400-650 km the  $\beta$ -phase will transform to the spinel phase,  $\gamma$ ; at  $\sim$ 650 km the spinel most likely transforms, by a disproportionation reaction, into the perovskite and rocksalt structures which seem to persist deep into the lower mantle.

The temperature of the olivine to  $\beta$ -phase transformation is estimated to be 1,650 °C, based on the mantle temperature distribution <sup>10</sup>. Taking these P, T conditions to be the optimum

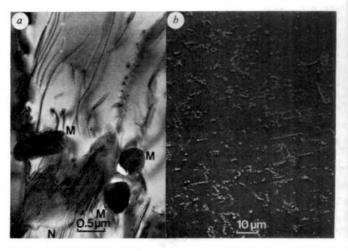


Fig. 3 Iron-nickel precipitates (M) in sample B. a, The precipitates are located in the edge dislocation sub-boundaries but they are absent from the cross-array of screw dislocations at N. b, SEM showing the clustering of the metallic precipitate as well as the crystallographic alignment: sample B.

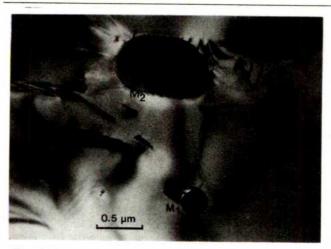


Fig. 4 The smaller metallic precipitate at  $M_1$  lies within the subgrain while the larger precipitate,  $M_2$ , is attached to the (100) edge dislocation sub-boundary-according to the postulated coarsening mechanism,  $M_1$  is dissoving while  $M_2$  is growing: sample B.

for the growth of the iron (nickel) precipitates in olivine the precipitate sizes expected after 10° yr coarsening are 20, 15, 2 and 1 mm using the values of V given above. These calculations, based on our experimental results, use the Gibbs-Thompson equation 14,16 assuming: (1) the precipitate/matrix interfacial free-energy and, (2) the equilibrium solubility of Fe in the matrix (for large precipitates), are constant over the P-T ranges used. Although there are no data for the Fe diffusivity in the  $\beta$ ,  $\gamma$ , perovskite and rocksalt structures, it is unlikely that the coarsening rates will differ by more than one or two orders of magnitude from the values calculated according to the olivine data, provided appropriate P, V and T values are used in the diffusion equation. For example, based on the olivine data, in 109 yr, at 2,000 km (corresponding to 2,500 °C and 100GPa-1 Mbar), the precipitate size would be about 5 mm for a V of 1 cm<sup>3</sup>. One other variable is the  $f_{O_2}$ . Intrinsic  $f_{O_2}$  measurements21 on spinels from peridotites indicate a wide range from just above the iron-wustite buffer to the OPI buffer (Fig. 1). for will certainly influence the diffusion coefficient but as with Ni diffusion in NiO (ref. 22) the effects may be small for very low foz.

Although solid-state diffusion may not lead to any large particles of iron-nickel, it has recently been proposed that the incorporation of Fe (and Ni) in refractory metal particles in the Allende meteorite occurs as a postnebular condensation-reduction reaction<sup>23</sup>. In addition, the condensates from a nebular gas accrete to form planetesimals and ultimately the planets. Unless the accreting body is completely molten, it will be necessary to transfer iron (and nickel) from a solid silicate phase into the metallic phase which may be molten (see below); in addition, other light elements including sulphur, oxygen, silicon and hydrogen may diffuse into the metallic phase. The mechanism of formation (nucleation) and coalescence (coarsening) of the metal will be critical steps in the segregation/ sinking stage of the metallic phase into the formation of the core, regardless of whether the overall accretional processes are described as homogeneous<sup>10</sup> or heterogeneous<sup>24,25</sup>.

For a homogeneous transformation involving GP-like zone formation, the diffusivity pathways are exceedingly small, possibly no greater than 200 Å between zones. However, if the zones are now liquid instead of solid, the coarsening process would not involve the slow solid-state, Ostwald ripening process but a modified version of hydrodynamic dispersion in which the mass transfer occurs by molten iron convecting along subgrain and grain boundary channels. This mass transfer process has effective diffusivities several orders of magnitude greater than normal diffusivities26. In addition our observation shows that in the very early stages of the formation of metallic particles, nickel is preferentially removed from the silicate phase. To avoid the

excessive depletion of siderophile elements implied by such a process, advocates of the homogeneous accretional theory propose the sinking of large metallic 'drops' that originate close to the surface of the Earth<sup>27</sup> or at 1,500-2,000 km depths<sup>10</sup>. The former model implies that olivine would be the stable phase while in the later proposal the high-pressure polymorphs would be more appropriate. However, at such high pressures the ferrous ion in the silicate phase is expected to disproportionate into the ferric state plus metallic iron 28. In any case, whether the formation of a metallic phase is by a reduction reaction or disproportionation, a coarsening reaction is implied and our observations for the solid state and those relating to the molten state29, indicate that large-sized precipitates would not form. A further complication arises because there is no agreement as to the relative abundances of siderophile elements (contrast refs 10 and 30), leaving the metal-silicate partitioning a highly controversial issue in models of Earth and Moon formation.

The boundary conditions imposed by the coarsening rates of solid and/or liquid precipitates in a solid silicate phase indicate that the evolution of the mantle and core involves a multi-stage processing of primitive material. Relicts of these primitive microstructures would be rare<sup>31</sup> because of subsequent modifications occurring during phase transformations<sup>32</sup> and mantle convection33. Although the solid-state growth rates reported here are insignificant for the large scale phenomena needed for core formation, they are certainly adequate to account for the micrometre size metallic phases and exsolution lamellae reported in lunar rocks<sup>2,34,35</sup> and meteorites<sup>36</sup>.

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# Structural features in ethanol-water mixtures revealed by picosecond fluorescence anisotropy

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When an ensemble of molecules is excited with polarized light an anisotropic orientational distribution with respect to the transition dipole moment is produced. This anisotropy can decay in time due to the rotational motion of the molecules and consequently leads to depolarization of the fluorescence 1-6. The rate of this rotational motion has been successfully predicted from hydrodynamic theory. How much the rotational relaxation depends on molecular geometry and how much on specific solvent-solute interactions has been studied by picosecond spectroscopy<sup>1-6</sup> and other techniques<sup>7-9</sup>. In all cases so far reported, the rotational behaviour seems to be accounted for by the Debye-Stokes-Einstein (DSE) equation  $\tau_R = f/kT$ . This relates the rotational relaxation time  $au_{
m R}$  (inversely related to the rotational diffusion coefficient) to the frictional coefficient, f, which is proportional to the product of the shear viscosity, the molecular volume and a constant dependent on the 'stick' or 'slip' boundary conditions<sup>3,10,11</sup>. We report here, however, that large deviations from DSE behaviour have been observed in the rotational diffusion of the dve cresyl violet in ethanol-water mixtures. Different rotational relaxation times are observed in solutions of the same viscosity but differing composition. This behaviour can be rationalized using previously proposed models for water-ethanol mixtures.

Most information previously obtained from fluorescence depolarization data has been concerned with solvent-solute interactions rather than with the solvent itself<sup>5,6</sup>. Figure 1 shows a plot of the rotational relaxation time of the dye cresyl violet (3,7-diamino-1,2-benzphenoxazine chloride) against relative solvent viscosity  $(\eta/\eta_0)$  for ethanol-water mixtures<sup>12</sup> (points 1-11 in order of increasing ethanol content) and for acetone, methanol and propan-1-ol (points 12-14 respectively). Also shown is the line calculated from DSE theory for cresyl violet at the 'stick' boundary condition. The molecular shape and volume were calculated from van der Waals' radii. The composition ions of the solutions are shown in the legend to

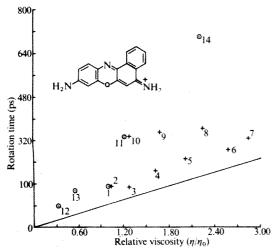


Fig. 1 Rotational relaxation time of cresyl violet (in ps) plotted against relative viscosity  $(\eta/\eta_0)$  for water-ethanol mixtures (points 1-11) with ethanol mole fractions of 0.0, 0.002, 0.024, 0.051, 0.079, 0.132, 0.207, 0.470, 0.706, 0.950 and 1.0. Acetone, methanol and propan-1-ol are points 12-14 respectively. The solid line has the slope calculated for DSE behaviour of cresyl violet at the 'stick' boundary condition.

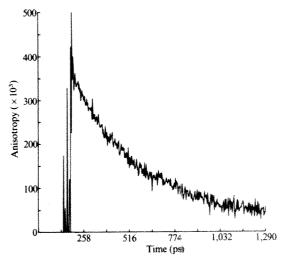


Fig. 2 The decay of the fluorescence anisotropy r(t) for cresyl violet at 770 nm in the 0.470 mole fraction ethanol solution, calculated from the fluorescence intensity parallel  $(I_{\parallel})$  and perpendicular  $(I_{\perp})$  to the excitation polarization using the formula  $r(t) = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$ . The anisotropy is related to the rotational relaxation time  $\tau_{\mathbf{R}}$  for a sphere by  $r(t) = r_0 \exp(-t/\tau_{\mathbf{R}})$ . For molecular shapes approximated to ellipsoids the formulae for r(t) given in refs 3 and 4 were used.

Fig. 1; all the experiments were carried out at 294K. The fluorescence of cresyl violet was measured with picosecond time resolution using a frequency up-conversion spectrometer<sup>6</sup>. The fluorescence anisotropy was calculated from the normalized fluorescence decays measured with polarization parallel and perpendicular to that of the exciting light. An example of the fluorescence anisotropy decay is shown in Fig. 2. The dye concentration was  $10^{-4}$  M in all the solutions and is low enough to avoid modification of the bulk properties of the solution.

The most noticeable feature of the rotational relaxation data are the dual values at identical solvent viscosities. We can identify two regions with which to explain the observations. First, in the high water region (points 1-7) DSE behaviour is observed and the gradient of the line of measured  $\tau_R$  against relative viscosity is the same as that predicted from the molecular volume. Second, after the maximum ethanol-water solution viscosity is reached (points 8-11) the rotational relaxation time decreases only slightly until the value for pure ethanol is reached, even though the relative viscosity changes by a factor of greater than 2. Ethanol-water mixtures show large positive deviations from Raoults law and the viscosity is a maximum at a mole fraction of 0.25 ethanol. We have observed similar behaviour in other alcohols which will be reported elsewhere.

The difference in rotation time of cresyl violet between pure water and ethanol has previously been explained by the specific interaction of ethanol with the amino groups on cresyl violet. This increases the effective volume of the molecule thus making molecular rotation slower. This argument, when extended to the other alcohols is the reason for their rotation times being well above the calculated 'stick' line, the solid line in Fig. 1. In water it seems that there is little interaction with the cresyl violet, presumably due to strong water-water interactions. In the related dye oxazine-1 (3,7-bis(N,N-diethylamino) phenoxazine perchlorate) the amino groups are ethylated, hence no interaction can occur at this site with the alcohols; consequently, the rotation times in alcohols and alcohol-water mixtures fall just below the line calculated for 'stick' limit behaviour 13.

Although the DSE theory was originally developed for large bodies moving in a continuum it seems, nevertheless, to be applicable to the description of small molecules moving in slightly smaller discrete solvents<sup>6,10</sup>. One reason for this would seem to be that the motion of the solvent is considerably faster than that of the dye molecules. For instance, a correlation time of 2.5 ps for the protons in water and 8 ps for the methyl protons in pure t-butanol has been reported<sup>14</sup>. The dye molecule, on the

other hand, rotates in  $\sim 150$  ps in pure water and 330 ps in pure ethanol. As the local environment around the molecule is changed many times during its rotational decay time the solvent effectively presents a continuum in which the solute moves. However, in the case of alcohol-water mixtures it seems that some structural features are maintained for long enough to affect the rotation of the solute.

Our results support models of 'hydrophobic hydration' deduced from thermodynamic and spectroscopic data 15-17. It is suggested that in the region of increasing viscosity, up to  $\sim 0.2$ mole fraction ethanol, the ethanol may be considered to lie in interstices in a three-dimensional water 'structure'. The hydrophobic part of the ethanol is surrounded by 'cages' of water although it does not interact significantly with these surrounding molecules<sup>14</sup>; the hydroxyl group, however, replaces one of the framework water molecules. The altered reorientational behaviour of the water molecules on adding ethanol is due to the enhancement of water-water interactions between these cages. The water-ethanol interactions play a smaller part<sup>18</sup>. At higher alcohol concentrations, above  $\sim 0.2$  mole fraction ethanol the ethanol can no longer be contained in the basic water structure and this disintegrates; breaking of the water-water hydrogen bonds leads to a decrease in viscosity. A more detailed and comprehensive discussion of the properties of water-alcohol mixtures is given elsewhere16

At low mole fraction of ethanol our results suggest (points 2-7, Fig. 1.) that the ethanol is surrounded by water as no specific interaction of the ethanol with the cresyl violet is observed; that is the variation of the rotational relaxation time with relative viscosity is that predicted for cresyl violet alone. Above the maximum viscosity when the ethanol is no longer contained in the water cages it is available for solvation of the cresyl violet. This is shown in the data by an increase in the measured rotational relaxation time when the solvent viscosity is decreasing (points 7-9). If the rotational diffusion rate at high ethanol concentrations were due to cresyl violet-ethanol interactions alone then  $\tau_R$  would be constant at 330 ps over the viscosity range.

The small variation in the rotation time over the viscosity range 2.8-1.2 indicates that in this composition range the cresyl violet is probing small pools of ethanol contained in the water. The slightly larger rotation times above 0.3 mole fraction (points 8 and 9) could be due to the small pool size at this composition as they are surrounded by water. This effect is analogous to that occurring for small pool sizes in reversed micelles where an increased viscosity is observed<sup>19</sup>. As the pools become larger the effect of the surrounding water is reduced and the rotation time tends towards that in pure ethanol. The possibility that the anisotropy decay probes free ethanol as well as water-ethanol cages was also considered. This would require the anisotropy to be a combination of two rotational decay times but analysis of the data in this manner will not support this supposition indicating that only a single species is present. Thus, due to its ability to interact preferentially with one component of the mixture cresyl violet can probe some of the structural features of alcohol-water mixtures that manifest themselves over a relatively long time, that is a few hundreds of picoseconds.

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### **Subduction of the Cocos** plate in the Mid America Trench

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The Mid America Trench, off the Pacific coast of southern Mexico and central America, is the site of northeastward subduction of the Cocos oceanic plate under the North America and Caribbean plates1 (Fig. 1). In Guatemala, the North America and Caribbean plates are separated by the Polochic-Motagua left-lateral strike-slip faults (Fig. 1). The trench itself appears to be divided into two distinct sections at its intersection with the Tehuantepec Ridge. To the south the margin of the trench is wide and consists of a well developed shelf basin<sup>2</sup> bounded on the east by a huge volcanic cordillera parallel with the trench axis at a distance of ~200 km. In contrast, to the north, the margin is narrow and devoid of shelf basin; landwards, the trans-Mexican volcanic belt trends oblique to the trench. This volcanic chain would be anomalous if it were related to the subduction of the Cocos plate off southern Mexico. In 1979, as part of the International Phase of Ocean Drilling (IPOD) of the Deep Sea Drilling Project, Legs 66 and 67 respectively explored the northern (off Acapulco, Mexico) and southern (off San Jose, Guatemala) section of the Mid America Trench<sup>3-6</sup> (Fig. 1). Since then short topographical surveys of the trench have been carried out by RV Jean Charcot<sup>8</sup> using the sea-beam technique. We report here that both sets of data, together with UTMSI multichannel seismic profiles 10,11 can be used to reconstruct the processes of subduction along the Mid America Trench.

Leg 67 (refs 3, 4), in the southern section seems to include the first occasion that a drill-hole in an oceanic trench reached the oceanic basement. The sharp contrast between the Upper Cretaceous sequence at the base of the continental slope and the subducting Lower Miocene Cocos plate beneath it suggests that in the region of Leg 67, subduction is not accompanied by accretion. Data from Leg 66 (refs 4, 5) in the Northern section of the trench show an upslope increasing age of turbidites on the continental wall (Pleistocene up to Middle Miocene) indicating accretion. In the absence of more representative drilling data from the northern and southern sections, however, the results of Leg 67 could be explained by the slumping of a large block of Cretaceous material and those of Leg 66 by the trapping of coarse sediments in suspended ponded basins.

The results of the sea-beam surveys (Fig. 2a, b) confirm the general features of the Mid America Trench; they also give new data that clarify the mechanism of subduction in the northern and southern sections. The most striking feature of the data is that the Cocos plate is marked, in the neighbourhood of the trench, by elongated ponds and swells trending 130°-140° N, that are clearly oblique to the mean 110°-120° N direction of the trench (Fig. 2a, b). This horst and graben pattern, already known from previous seismic records  $^{10,11}$ , is thus not as parallel

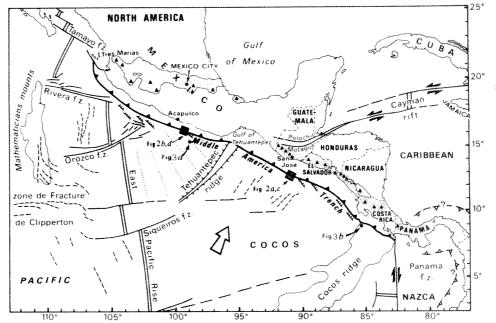


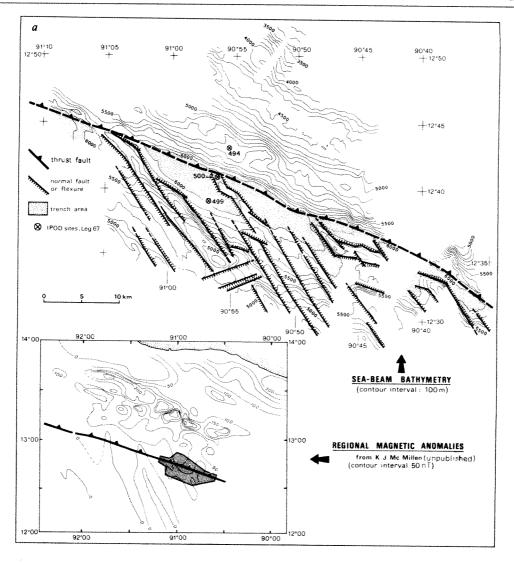
Fig. 1 Tectonic framework of the Mid America Trench.

to the trench axis as was assumed. In the Leg 66 area, at the mouth of the very active Ometepec canyon<sup>11</sup>, the Cocos pattern disappears towards the trench under a thick pile of turbidites (Figs 2b, d). In contrast, in the Leg 67 area, Cocos structures totally control the trench morphology. Indeed the trench consists of a succession of diamond shapes (corresponding to graben structures) and bottle-necks (corresponding to horsts) (Figs 2a, c). This is consistent with the drilling results at hole 500, just at the base of the continental slope, where a normal fault was suspected between Pleistocene trench sediments and Miocene chalk and where oceanic crust was reached 150 m above the same material in the trench<sup>3,4</sup>. Most probably hole 500 is just at the western edge of a Cocos horst. What about the origin of this oblique pattern? Is it an en échelon faulting due to oblique subduction or is it related to the Cocos plate internal grain? The latter solution is favoured by the clear parallelism observed between the horst and graben pattern and all known magnetic anomalies near the trench (refs 11, 12 and K. J. McMillen, unpublished data) (Fig. 2). Thus we consider that this structural pattern is controlled by inherited faults formed at the East Pacific Rise. Indeed it has been shown 13,14 that the genesis of oceanic crust along the East Pacific Rise is accompanied by dense normal faulting and fracturing, parallel with the spreading axis. These faults become inactive  $\sim 10 \text{ km}$  off the rise<sup>13,14</sup>; however, they still exist as mechanical discontinuities. The bending of the Cocos plate in the subduction zone induces surficial tensional stress that preferentially reactivates these older fractures. The easier the reactivation, the smaller the angle (20-30°) between the mean direction of the trench (110°-120° N) and that of inherited fractures (130°-140° N). Such a reactivation process has already been proposed—with far less evidence—for the Nazca plate along the Peru-Chile trench<sup>15</sup>. The oblique subduction hypothesis can be rejected first, because the direction of convergence between the Cocos and the North America-Caribbean plates is well known 16,17, about 30° N that is quite perpendicular to the trench along most of its length; and second, because off Salvador and Nicaragua where the trench trends 140° N and the subduction is thus oblique, our sea-beam survey shows the horst and graben pattern parallel with the trench.

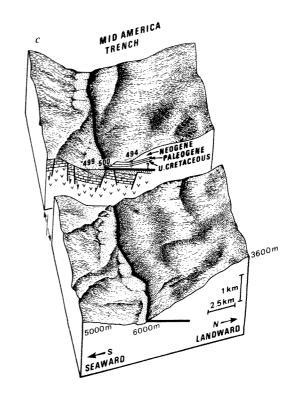
The sea-beam map shows that in the Leg 67 area (Fig. 2a), the Cocos structures reach the base of the continental wall without disturbing it. The main thrust fault of the subduction zone is apparently between the continental slope, where Upper Cretaceous sediments were drilled in hole 494 and a Cocos horst where the Lower Miocene oceanic crust was reached in hole 500. Thus, off Guatemala, neither the drilling results nor the sea-beam surveys indicate that a classical accretionary prism<sup>2</sup>

has developed. The presence at the base of the continental slope of such a sequence involving Upper Cretaceous and Tertiary in good stratigraphical order still needs explanation. It could be part of a large block slumped down the slope in recent times; however, sea-beam data (Fig. 2a) indicate that the base of the innerwall is perfectly rectilinear and that no scar is present. Anyway, the Upper Cretaceous sequence could belong to a block slumped in pre-Pliocene or Miocene times: that would mean that no accretion has occurred at least since then. Finally, the whole sequence of hole 494 could be considered as in situ implying no accretion for more than 70 Myr or that tectonic erosion removed younger accreted terranes. Only new drilling will eventually solve the problem. In the Leg 66 area (Fig. 2b, d) the infilling of the trench means that the contact itself between Cocos structures and the innerwall is not visible. However, the sea-beam map shows a more or less continuous ridge in the middle of the trench. Such a central crest was first described off Costa Rica, at the very end of the trench, from submersible observations<sup>18</sup>. Off Southern Mexico the ridge has a maximum relative height of 100 m and is flanked on its seaward side by an elongated depression in which anastomosed channels of the Ometepec deep-sea fan seem to converge. Central ridge and channel define the limit between a relatively smooth outer trench and an undulated and uplifted inner trench. Sea-beam data confirm previous interpretations from UTMSI multichannel seismic reflection lines crossing the same area11. Thus the central ridge appears to be located above the most recent emergence of the Benioff plane. In the inner trench, turbiditic reflectors are gently folded; in particular, south-east of the Ometepec canyon mouth both reflection data and sea-beam maps show the inner trench as a synformal depression (Figs 2b, d). Upslope, on the continental wall, the same ridgeand-basin morphology is present but shows far more contrast and is mature. Site 488, drilled on the first ridge at the base of the innerslope, reached tectonized turbidites 400-500 m higher than the present-day trench turbidites<sup>5</sup>. Finally, although no tectonic repetition has been confirmed by Leg 66 drillings, the data strongly suggest that trench sediments are being accreted to the Mexican margin off Acapulco<sup>5,6,11</sup>

Regarding the Cocos plate sea mounts related to off-axis volcanism are locally abundant and prevent development of a regular tectonic pattern. One of these sea mounts already mapped at 15°27'N-98°31'W off Mexico<sup>11</sup> is revealed by seabeam bathymetry as a nice collapse-pit volcano about 6.5 km in diameter (Fig. 3a). Other sea mounts are smaller regular cones belonging to the Cocos Ridge off Costa Rica and entering the subduction zone without apparent deformation (Fig. 3b).



Our sea-beam surveys confirm that at least two types of subduction are acting along the Mid America Trench. Off Guatemala (Leg 67 area) Cocos crust and sedimentary cover, as well as trench infilling are totally subducted beneath the continental margin. Some kind of tectonic erosion may have occurred to explain the atypical Upper Cretaceous sequence drilled at the base of the innerwall. In contrast, off Mexico (Leg 66 area) the thick pile of trench turbidites is actively accreted to the continental margin. The dual aspect of the innerwall does not appear on the seaward side of the trench. Close to the subduction zone, in both areas, the Cocos plate shows the same horst and graben pattern superimposed on inherited fractures formed at the East Pacific Rise. The fanning of magnetic anomalies on the Cocos plate 19 together with IPOD results indicate that the crust being subducted is progressively older in a southeastwards direction. The Tehuantepec Ridge breaks this continuity20, but does not mark a boundary between two different crustal provinces. Thus the Cocos plate does not seem to have a role in the type of subduction. Variability of other parameters could account for that phenomenon. For example, the convergence rate progressively increases from north to south<sup>17</sup> (being ~7 cm yr<sup>-1</sup> off Mexico and 10 cm yr<sup>-1</sup> off Guatemala); the dip of the Benioff zone is lower along the northern section of the trench 16; the trench fill is generally more important off Mexico than off Central America21. The last parameter is probably critical in the subduction. However, in the Mid America Trench, the terrigenous input closely depends on the morphostructure of the margin: on the northern section the narrow shelf is cut by numerous canyons that allow direct



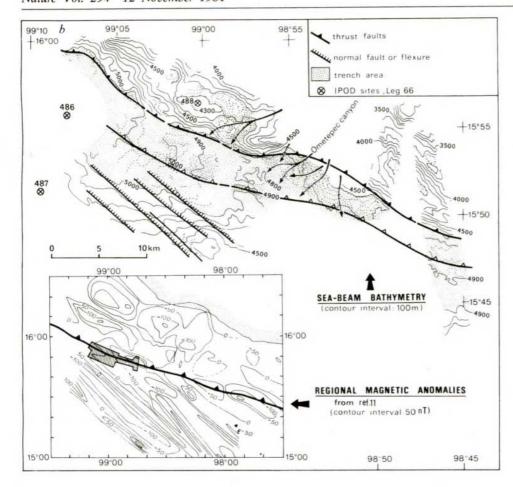
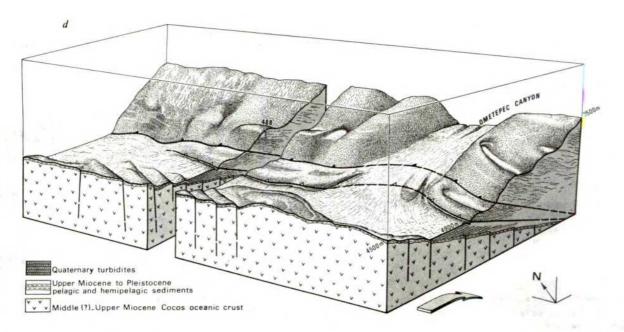


Fig. 2 Sea-beam map of a, the Leg 67 area and b, the Leg 66 area. (The original 10-m contour interval has been condensed to 100 m.) Schematic diagram of c, the Leg 67 area (after ref. 9) and d, the Leg 66 area.



transport to the trench of the clastics supplied by wide mountainous areas in the background (Sierra Madre del Sur); to the south, off Central America, the fluviatile input is far less important and most terrigenous material is trapped in the wide shelf basin. We know these two kinds of active margin belong to distinct plates (that is, the North America and the Caribbean plates). The boundary between both margins is located in the Gulf of Tehuantepec<sup>2,20</sup> that is exactly in the prolongation of the Polochic-Motagua fault system (Fig. 1). Thus, the northern and southern margins of the Mid America Trench directly reflects the geodynamic evolution of their own plate. For example, the

Mexican margin has experienced in Lower Tertiary times tectonic truncation and erosion that scraped off all Mesozoic accreted terranes as well as part of the older basement 11,12,22,23. In contrast, along the Central America margin a fore-arc basin has developed at least since the Palaeocene; moreover, a belt of Mesozoic ophiolites is still preserved on the margin 2,10. Regarding the present, tensional stress is responsible for the genesis of all known graben (Guatemala City, Nicaragua, and so on) south of the Polochic-Motagua fracture zone, while north of this boundary compressive deformations are occurring 24. We conclude therefore that not only simple parameters (such as

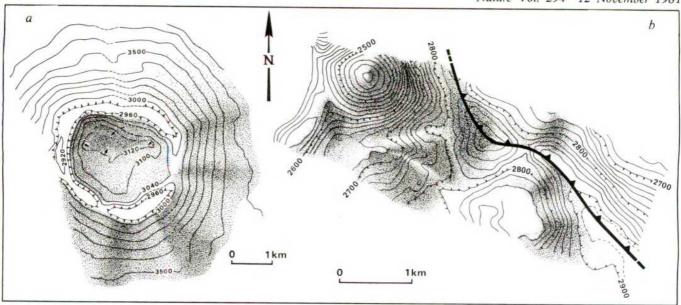


Fig. 3 Collapse-pit volcano off Mexico (a) and sea mounts on the Cocos ridge (b). See location on Fig. 1. Contour interval, 100 m on a; 20 m on b.

convergence rate and trench fill) account for the variation of the subduction process but also the properties (such as structure and kinematics) of the overriding plate. The Mid America Trench is

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a useful area for investigating the problem and for determining how far the upper plate governs the dynamic of the subduction

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# Peralkaline volcanicity on the Eurasia Basin margin

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The Kap Washington Group of post-Palaeozoic explosive volcanic rocks was discovered in 1969 on the north coast of Greenland. Although there have been uncertainties regarding their age and chemical character, they have featured prominently in geotectonic reconstructions of the Arctic regions-in recent interpretations as products of the Yermak hot spot, generated on the Nansen spreading axis during the opening of the Eurasia Basin. We present here new evidence which confirms the volcanicity as end-Cretaceous in age and of peralkaline type. We show that a direct connection with the Yermak hot spot is improbable and infer that the volcanic rocks were generated in a continental extensional rift environment before the break-up of the Laurasian plate in the Arctic. Their age helps to constrain the timing of this poorly understood event.

Aeromagnetic surveys conducted in the past decade 1-3 have established that the Eurasia Basin developed in Cenozoic time by ocean-floor spreading on the Nansen (Gakkel) Ridge, which detached an elongated continental fragment (the Lomonosov Ridge) from the Barents margin of the Eurasian plate (Fig. 1). There are problems concerning both the geometry and chronology of this phase of spreading. The geometrical problems arise from the uncertainties which still exist concerning the origin of the Amerasia Basin and Alpha Ridge2.4 and also the constraints imposed by the limited displacement permissible on the Nares Strait line5 on the geometry of opening of the Labrador Sea and Baffin Bay. Although recent magnetic anomaly superposition studies 1-4,6-9 have shed much light on the displacement patterns of the individual ocean basins around Greenland, it is clear that rigid-plate models do not lead to a complete geometrical solution and that within-plate strains must be important. Such strains are evidenced by the belt of Tertiary which extends from the Canadian Arctic (Eurekan Orogen 10,11) across northern deformation Archipelago Greenland 12,13 to Svalbard (West Spitsbergen Orogen 14).

The main chronological problem posed by the evidence from the Eurasia Basin is the time at which spreading was initiated. In dating magnetic anomalies and in relating on-land geological evidence to the history of the oceans, it is essential to use a recent polarity time scale. We adopt that of Hailwood et al. 15, noting that the commonly used Heirtzler et al.16 scale assigns ages to magnetic epochs in the late Cretaceous and early Tertiary which are too high by  $\sim 10\%$ . The oldest ocean-floor anomaly recognized in the Eurasia Basin is 24 (52 Myr, early Eocene) and a broad negative anomaly exists over the 50-100 km wide zone between it and the Lomonosov and Barents continental slopes, in which there is space to accommodate anomalies 25-28 (ref.

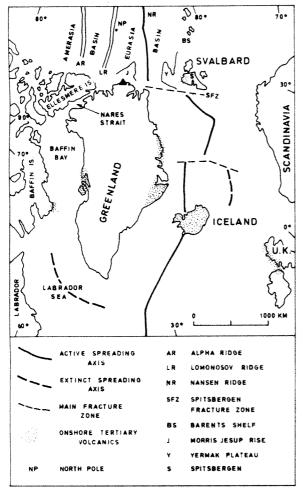


Fig. 1 Index map of the North Atlantic region. ▲, The location of the Kap Washington Group of volcanics in northern Greenland.

2). The crust flooring this zone could thus represent subsided continental crust, oceanic crust generated during a pre-anomaly 24 quiet period, or oceanic crust generated during the time of anomalies 25-28 (55-63 Myr, Palaeocene), whose magnetic expression was subsequently erased. This uncertainty should be seen in the light of the recently suggested history of the Amerasia Basin where seafloor spreading anomalies 34-19 (Cretaceous to mid-Eocene) are deemed identifiable between the Lomonosov Ridge and Alpha Ridge<sup>4</sup>.

Notable features in the present context are the aseismic submarine plateaus off northern Greenland (Morris Jesup Rise) and Svalbard (Yermak Plateau). These are thought to be constructional features produced by enhanced basaltic discharge from a Yermak hot spot situated at the junction of the Nansen Ridge and the Spitsbergen Fracture Zone<sup>3</sup> (Fig. 1). High-amplitude magnetic anomalies are associated with these plateaus, and they are bracketed by ocean-floor anomalies 13-18 (35-40 Myr, late Oligocene-early Eocene). This is thought to represent the period of maximum hot spot activity after which the subsiding plateaus were rifted apart by continued spreading. It has been proposed<sup>3</sup> that the volcanic province on the north coast of Greenland (Kap Washington Group<sup>12,17</sup>) is an early manifestation of this hot spot activity. If this were the case, the age of the volcanic rocks would provide an indication of the time of initiation of the hot spot volcanism, and thus of the onset of spreading in the Eurasia Basin. However, our data on the age and chemical character of the Kap Washington Group volcanics reported here demonstrate that the eruptives represent the products of peralkaline rift volcanism that predates the spreading history of the Eurasia Basin and thus the eruptives are unlikely to be directly associated with the Yermak hot spot.

The Kap Washington Group volcanic rocks outcrop at about 83°30'N on the north coast of Greenland at the edge of the

North Greenland fold belt, where they have a chronological position postdating the mid-Palaeozoic diastrophism of the fold belt but predating its Tertiary reactivation. Limited field observations in  $1969^{12.18}$  have been supplemented by field work in  $1979^{19}$  and  $1980^{20}$ . The outcrop of the volcanic rocks is limited to  $\sim 150 \text{ km}^2$  (Fig. 2), but considerable offshore extension may be indicated by the Morris Jesup Rise magnetic anomaly 3.21, although the nature of the rocks responsible for the anomaly is not known.

The extrusive volcanic rocks form southeasterly inclined sequences; they are non-metamorphic but locally show shearing and crushing and in places cleavage. The thickness of the exposed volcanic sequence is at least 5 km. It is composed of prominent pyroclastic rocks, including coarse breccias, finely bedded airfall tuffs, accretionary lapilli tuffs and welded ashflow tuffs, as well as both basic and acidic lavas. Some intrusive rock types are present, mainly felsic dykes and small plugs. The upper boundary of the volcanic sequence is a thrust contact with Lower Palaeozoic metasediments of the fold belt; thrusts also disturb the volcanic succession and bring in wedges of Carboniferous and Permian strata of the Wandel Sea Basin (Fig. 2).

Hitherto unpublished chemical data for material collected in 1969<sup>18</sup> and new petrographical data for recently collected material<sup>20</sup> demonstrate that the Kap Washington Group volcanics represent a peralkaline suite. The 1969 material is mainly rhyolitic and is difficult to characterize chemically; on a silica/alkali plot the rocks fall, not unexpectedly, in the subalkaline field. The single available analysis of a basic flow is thoroughly alkaline. The 10 analyses of rhyolitic material (SiO<sub>2</sub> range 66-75%) show appreciable alteration of major element composition and there is a marked variation in the Na<sub>2</sub>O/K<sub>2</sub>O ratio (total alkalis  $\sim 8.5\%$ ). CaO varies from 0.02 to 1.83 and Al<sub>2</sub>O<sub>3</sub> is between 10 and 18%. However, two samples show normative acmite—a clear indication of peralkalinity—and the trace element composition, particularly with respect to the relatively high values of Nb, Zr and Y, conform to those values normal for peralkaline rocks<sup>22</sup>. Petrographical results indicate that basalts, riebeckite trachytes and riebeckite comendites or pantellerites are the prominent lava types. Some basalts have a strongly developed flow texture and may overlap into the compositional field of hawaiites. The trachytes contain anorthoclase, ill-formed riebeckite and quartz in the flow textured groundmass. The more siliceous lavas have a variable riebeckite content and include flow banded and spherulitic varieties. The presence of the soda amphibole riebeckite establishes the peralkaline character of the Kap Washington Group.

The Kap Washington Group volcanic rocks conformably overlie black shales and sandstones which contain fragmentary plant debris. While no microfossils have been isolated from the sediments, a few angiosperm leaf fragments indicate a mid-Cretaceous or younger age. The available angiosperm leaf fragments are not determinable at either genus or species level. One small, nearly entire leaf specimen is a very primitive morphological type appearing in the Lower Cretaceous (Albian) floras of the eastern USA and Portugal (Proteaephyllum Fontaine and similar form genera). Another is a leaf fragment of an advanced pinnate angiospermous leaf type with percurrent nervils appearing in the Upper Cretaceous and continuing through the Tertiary. The primitive leaf type makes a Cretaceous age probable for the shale in question. The advanced leaf type does not permit an age older than mid-Cretaceous. However, with the very limited material available this can only be a tentative age.

Other black shales are interbedded with the pyroclastic rocks some 3 km above the base of the volcanics and several samples have been subjected to palynological investigation. The microfossils recovered are generally in a very poor state of preservation and few can be identified. Bisaccates, smooth-walled triradiate spores and some distinctive angiosperm pollen grains, identified as species of Aquilapollenites, Mancicorpus and Triprojectus, have been recorded, but all other identifications are questionable. Fortunately, the named genera have restricted

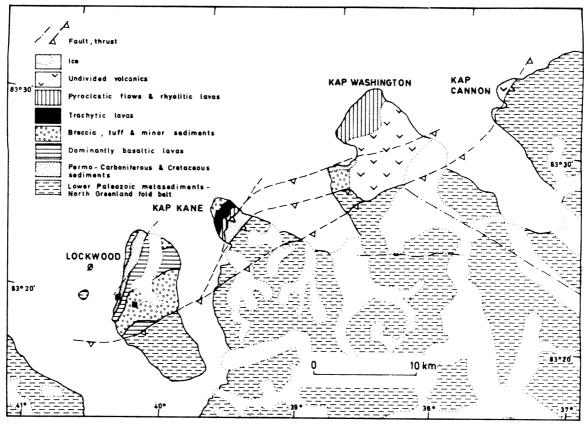


Fig. 2 Geological map of the total outcrop of Kap Washington Group volcanics. , Localities of the fossiliferous shales.

stratigraphical ranges and a late Cretaceous Campanian or Maastrichtian age is indicated; that is 75-65 Myr on the stratigraphical time scale. The upper limit is in close agreement with an earlier Rb-Sr whole-rock age of  $63 \pm 2$  Myr on five rhyolitic samples23, a result which had previously been regarded as unreliable on technical grounds. An age for the volcanicity at, or just before, the Cretaceous-Tertiary boundary is adopted here.

The characteristic tectonic environment of peralkaline volcanism is one of crustal extension and attenuation, so that their most abundant development is in association with regional doming and rifting<sup>24</sup>. Evidence of crustal extension immediately preceeding the extrusion of the Kap Washington volcanics is provided by alkali dolerite dykes and sills<sup>13</sup>. These cut the early Cretaceous and Carboniferous-Permian sediments, as well as older rocks of the North Greenland fold belt, but fail to penetrate the Kap Washington Group volcanic rocks<sup>25</sup>. These dykes, like the volcanics, show alteration and some shearing due to Tertiary tectonism. K/Ar whole-rock determinations show a range of apparent ages, although the most reliable of these away from areas of known Tertiary tectonism lie between 82 and 66 Myr (ref. 26). The dyke swarms are regarded as late Cretaceous in age.

The composition of the Kap Washington volcanics illustrates that the tectonic regime in the Barents Shelf-Greenland region in late Cretaceous time was extensional, as indeed it was in the entire region from the Sverdrup Basin of Arctic Canada through northern Greenland to the northern Barents Shelf. The palaeontological dating of the Kap Washington Group reported here places the beginning of the active spreading in the Eurasia Basin as probably no earlier than the Cretaceous-Tertiary boundary (perhaps as early as 75 Myr). The various reconstructions of the Barents Shelf margin before the seafloor spreading 1,3,4,9,13 place Svalbard close to northern Greenland and it has been suggested that the Kap Washington volcanics and even the earlier basic dykes are manifestations of the Yermak hot spot3. On the grounds of both their age and composition, we would not relate the Kap Washington Group or the basic dykes directly to the Yermak hot spot; the dykes and

volcanics are late and end-Cretaceous in age, respectively, while the first manifestation of hot spot activity in the ocean floor is at anomaly 18 time (40 Myr, late Eocene). The geographical association of the alkalic rift magmatism and later hot spot activity may well have petrogenetic significance, but this problem must await petrological studies of the Kap Washington volcanics and also sampling of the hot spot rocks from the submarine plateaus. Further consideration of Cretaceous-Tertiary magmatic and tectonic events in northern Greenland and their relationships to the history of adjacent ocean basins is presented elsewhere1

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# Migration of late Cenozoic volcanism in the South Island of New Zealand and the Campbell Plateau

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In late Cenozoic volcanics of eastern Australia, Wellman and McDougall¹ have shown a remarkable southward migration pattern of major shield volcanoes of alkaline olivine basalt association which they relate to movement of the continental crust over a fixed mantle hot-spot. Contemporaneous tholeiite lava field provinces do not show a similar pattern. Following their general selection criteria¹, I believe a similar situation emerges for New Zealand examples. In the South Island of New Zealand and the adjacent Campbell Plateau and Chatham Rise are several late Cenozoic volcanic centres that rest on a peneplain in Mesozoic and Palaeozoic granites and metamorphic rocks²-³, which is partly mantled by late Cretaceous to mid-Tertiary shallow marine sediments⁴-⁵. These major centres of late Cenozoic alkalic volcanism show an eastward decrease in age from ~28 Myr to ~0.5 Myr, presumably reflecting movement of a linear mantle source with respect to the Pacific Plate.

The volcanic centres are widely scattered and three volcanic associations are present: (1) Several major shield volcanoes of alkaline olivine basalt association, for example, Banks Peninsula, Otago Peninsula, Auckland and Campbell and Chatham Islands. (2) Very minor and restricted tholeite, for example, Timaru Basalt. (3) A calc-alkaline andesite volcano at Solander Island (probably related to active eastward subduction of the Indian plate below Fiordland). Brief geological notes for these volcanoes are compiled in Table 1 and age distributions shown in Fig. 1. In defining the age trend, only data from major alkaline olivine basalt centres are used, that is, the first group described above.

Although the data are sparse, there is an eastward decrease in age for the volcanic centres, and although there are no marked chains of volcanoes, there seems to be a predisposition for

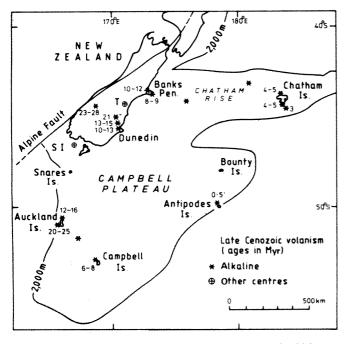


Fig. 1 Age data for centres of alkaline volcanism of mid-late Tertiary age in southern New Zealand and Campbell Plateau. SI, Solander Island; T, Timaru Basalt.

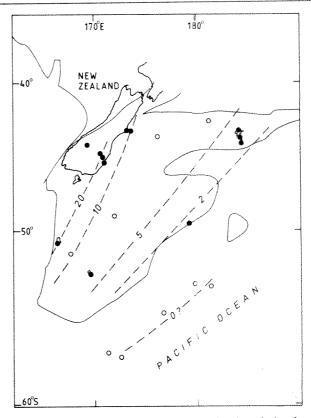


Fig. 2 Approximate locus of alkaline volcanism during late Tertiary on the basis of age data summarized in Fig. 1 from major volcanic centres dated (●). Possible submarine volcanic edifices, of similar age and type (○)<sup>4,5,29</sup> but undated.

volcanism to occur, at any one time, within a geographically restricted belt. The centres form neither arcs (as, for example, in the case of calc-alkalic volcanism) nor linear chains that arise from 'hot-spot' volcanicity. Using these age data Fig. 2 shows approximate isochrons which form a fan-shaped set beginning at 20 Myr ago, crossing the South Island in an NNE/SSW direction and rotating to a present NE/SW position close to the Campbell Plateau continental slope. This migration parallels the late Cenozoic movement of the New Zealand continental crust with respect to the active mid-oceanic spreading centre in the southwest Pacific (that is, an anticlockwise rotation of 60° about a pole approximately 65° S, 140° E).

Thus the location of the alkaline volcanism seems to be directly related to the passage of a mantle source below. At the surface there is evidence that the volcarism is locally controlled by major tectonic features: for example, Miocene NNE/SSW reverse faulting in east Otago<sup>6</sup> and recent NE/SW faults in the Antipodes Island and Campbell Plateau margin7. It is possible that the locus and style of faulting are themselves an expression of the passage of the hot mantle source below. A more speculative suggestion is that broad regional uplifts in the Oligocene-Miocene in Otago<sup>8</sup> and more recently on the Auckland/Campbell Island platforms4 might also reflect this process. The mantle source in this case cannot be regarded as a single 'hot spot'. similar to, for example, that which produced the Hawaiian volcanic island chain, or the slightly more diffuse source beneath the east Australian Cenozoic volcanoes. In the New Zealand-Campbell Plateau example, the mantle source responsible for the Cenozoic volcanism forms a relatively narrow (~100-200 km) linear belt, and is 1,000-1,500 km long throughout its course beneath southern New Zealand and the Campbell Plateau region. The movement of the mantle source corresponds closely to known late Cenozoic motion of the Pacific Plate with respect to the mantle. Hatherton indicated a similar migration pattern for the late Cenozoic-Quatenary calc-alkaline volcanic belts of the North Island of New Zealand formed at the Indian/Pacific Plate boundary. Using the poles and movement

Table 1	Cenozoic volcanic centres of the south-west Pacific region	

Area	Volcanic association	Rock type and trends	Age (Myr)	Ref.
South Island, New Zealand			0 . , ,	
Banks Peninsula				
Akaroa centre	Alkaline, shield volcano	Basalt-hawaiite-trachyte	89	
Lyttleton centre (i)	Alkaline	Hawaiite	10-12	11-13
(ii)	Alkaline	Basalt	5-8	11-13
Wanaka	Alkaline, dyke swarm	Basalt, lamprophyre	20-28	*
Dunedin	• •		#U#U	
Otago Peninsula	Alkaline shield volcano	Basalt-hawaiite-trachyte-phonolite	10-13	14-17
Waipiata province	Alkaline	Basalt, basanite	12-15.21	6*
Timaru	Tholeiitic, flood basalt	Basalt	2.5	18
Solander Island	Calc-alkaline volcano	Andesite	Pleistocene?	19
	Pacific/Indian Plate boundary		Tienstocone,	1,7
Campbell Plateau-Chatham Rise	, Pacific Ocean			
Campbell Island	Alkaline, shield volcano	Basalt-hawaiite-mugearite-trachyte	6.5-11	3, 20
Auckland Islands	Alkaline	magounte machine	0.5-11	3, 20
Ross Volcano		Basalt-mugearite-trachyte-rhyolite	12-16	20-26
Carnley Volcano		Basalt-trachyte	20-25	*
Antipodes Islands	Alkaline	Ankaramite, basalt	0.2-0.5	7
Chatham Islands		,	0.2 0.5	,
Northern centre	Alkaline	Limburgite	35	
	Alkaline, agglomerate vents	Limburgite	4-5	27, 28
Pitt Island centre	Alkaline, agglomerate vents	Limburgite-phonolite	2.6~5	,o
Chatham Rise		Basalt	Miocene-	29
			Pliocene?	

\* C.J.A., unpublished K-Ar data

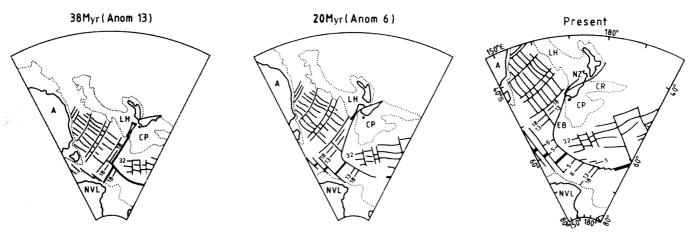


Fig. 3 Present day seafloor magnetic anomaly pattern and reconstructions of the south-west Pacific region 20 Myr and 38 Myr ago from Figs 13, 14 and 15 of ref. 30. A, Australia; CP, Campbell Plateau; CR, Chatham Rise; EB, Emerald Basin; LH, Lord Howe Rise; NVL, North Victoria Land, Antarctica; NZ, New Zealand.

rates deduced from seafloor spreading data, one can show that the mantle source responsible for the alkalic volcanism of the southern New Zealand region must now lie to the south-west of the Campbell Plateau margin and in the southwestern Pacific Basin. In this way a presumed 'zero' isochron is shown in Fig. 2 and it is intriguing that this lies close to prominent submarine features interpreted by Summerhayes<sup>4</sup> as possible volcanic seamounts. However, these have never been sampled or dated.

Similarly, the earlier history of the mantle source can be deduced from known mid-Cenozoic plate motions. The oldest and westernmost alkaline igneous activity is in west Otago, where a late Oligocene basaltic and lamprophyre dyke swarm occurs. Thus a mantle source must have originated there, or immediately to the west and before the late Oligocene.

It is likely that the long linear mantle source is related to a process of major reorganization of the Indian-Pacific-Antarctic plate motions in the southern New Zealand region in early-Oligocene times (~38 Myr; Fig. 3), and particularly to the formation of the Moonlight Aulacogen<sup>8,10</sup> at the southwestern margin of the Pacific plate. The development of this aulacogen controlled Oligocene sedimentation in the Emerald and Solander Basins (at the western margin of the Campbell Plateau) and the on-land extension northward into the Waiau Basin and Moonlight Tectonic Zone up to the Alpine Fault. The dimensions of the mantle source inferred from the pattern of volcanic centres would certainly be compatible with a linear tensional feature such as the Moonlight Aulacogen. The sub-crustal processes producing this aulacogen also possibly provided a mantle source for subsequent alkaline volcanism. However, immediately after this, a new phase of late Cenozoic seafloor spreading occurred in the southwestern Pacific (Fig. 3). Thus the continental crust of southern New Zealand and the Campbell Plateau began to move northwestwards over the mantle source and the subsequent alkaline volcanism is now expressed in a pattern of eastward migrating centres. The origin and generation of this new mantle source in relation to the early Cenozoic history is discussed in a new geophysical model of the region by Farrar, Adams and Dixon (in preparation).

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# Strontium isotopic resolution of magma dynamics in a layered intrusion

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Kalka is one of about 14 major layered mafic-ultramafic intrusions forming the Giles Complex in central Australia<sup>1</sup>. The stratigraphical section of the Kalka Intrusion passes from a basal pyroxenite zone (450+ m) through norite and olivine gabbro zones (3,500 m) to an uppermost anorthosite zone  $(800 + m)^2$ . Further resolution into 21 cyclic units is derived from repeated mineral crystallization sequences and cyclical variation in plagioclase and olivine compositions. A conventional interpretation would have a basaltic magma progressively fractionating as crystallization proceeded from mafic base to leucocratic top, with periodic resetting to less evolved states by fresh incursions of primary magma or by convectional overturn within a sealed magma chamber. However, the reconnaissance isotopic data reported here indicate development of a more complex open system. High initial <sup>87</sup>Sr/<sup>86</sup>Sr ratios in conjunction with normal 143Nd/144Nd ratios demonstrate massive contamination by country rock within the main part of the intrusion<sup>3</sup>. Furthermore, great variation in Sr initial ratios from 0.7049 to 0.7088 suggests substantial changes in magma composition beyond those induced by fractionation. Similar isotopic heterogeneity in the Newer Gabbros of Scotland<sup>4</sup> and the Bushveld Complex<sup>5</sup> has been attributed to variable contamination or the emplacement of magma batches of differing composition.

The present first detailed examination of the mechanism of isotopic variation in Kalka concentrates on the striking facies relationship between the norite and olivine gabbro zones. The norite zone (NZ) is the major component of the intrusion with a uniform clinopyroxene norite lithology reaching a maximum thickness of 3,500 m. Plagioclase composition shows an overall

upward variation from An<sub>75</sub> to An<sub>60</sub> broadly independent of the substantial separation into lower and upper subzones by the interleaved olivine gabbro zone. The much thinner olivine gabbro zone (OGZ) is totally enclosed within the body of the NZ and has a maximum thickness of 600 m and a strike length of 6.5 km (Fig. 1). It consists of an alternation of orthopyroxene gabbros similar to the rocks of the NZ and lesser olivine gabbro horizons, Plagioclase (An<sub>82-68</sub>) and olivine (Fo<sub>86-69</sub>) compositions oscillate as a function of stratigraphical position although co-crystallizing phases have been separated in the cumulate succession by differential gravity settling<sup>2</sup>. The facies link between the OGZ and NZ is expressed in the former by gradational boundaries defineable only on the appearance of olivine, and lateral interfingering of prominent bands into the NZ. The base of the OGZ is displaced to progressively higher stratigraphical levels towards the east and is laterally more extensive at these higher levels (a traditional 'transgressive' stratigraphical relationship in conventional sediments).

Initial 87 Sr/86 Sr ratios of rocks in a vertical section through the transition from the lower NZ to the OGZ are given in Table 1 and Fig. 2. The boundary is marked by a change from relative uniformity of initial ratio in the lower NZ at ~0.7078 to a cyclical variation in which values fall as low as 0.7068. The upper stratigraphical limit of the cycles lies beyond the sampled interval, but is probably the top contact of the OGZ. Eventually, the overlying section of the upper NZ saw a return to the initial ratio of its lower counterpart in the 0.70784 value of a reconnaissance gabbronorite sample. Overall, the continuous isotopic fluctuations of the OGZ can be described as a series of downward excursions from the general high value of the NZ. Overall variations in the total NZ itself reveal a small, gradual decrease in initial ratios from 0.7082 to 0.7073 from the base to the top of the zone. The petrological significance of the results is best seen in Fig. 2 through the correspondence of the onset of isotopic variation and the appearance of the olivine that defines the OGZ (olivine is rare in the 1,000 m of the underlying NZ). There is also an antipathetic decline in orthopyroxene, and a partial link between plagioclase and olivine compositional changes and the isotopic curve.

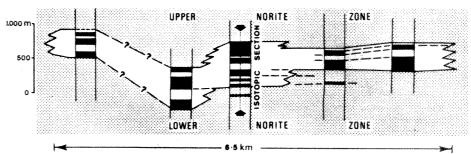
It is axiomatic that variations in Sr isotopic composition are due to physical processes and the only plausible explanation in this case is the mixing of materials with differing  $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$  ratios.

While the Nd-Sr study showed that primary basaltic magma and country rock granulite were the ultimate mixing components<sup>3</sup>, the absence of a discrete high-Si phase within the intrusion or other bodies in the Giles Complex points more to an interaction between primary magma (low <sup>87</sup>Sr/<sup>86</sup>Sr ratio) and contaminated magma (high <sup>87</sup>Sr/<sup>86</sup>Sr ratio) as the direct cause of isotopic fluctuations. Because the bulk of rocks within the intrusion show evidence of contamination, it is presumed that contaminated magma dominated the chamber and that primary magma was volumetrically minor and introduced from outside.

Given a low 87Sr/86Sr ratio in primary magma, any phase of decreasing initial ratio leading to an isotopic minimum (A, B, C of Fig. 2) can be attributed to a fresh incursion into the chamber and mixing with the established magma body. Incorporation of primitive magma could also lead to transient undersaturation and short bursts of olivine crystallization at that time (Fig. 2). Increases in initial ratio can be accounted for in two ways. If the

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Fig. 1 Schematic facies relationship between norite zone (stippled) and enclosed olivine gabbro zone (OGZ) in the Kalka Intrusion. Olivine-bearing units shown in black. Note increased lateral extent and eastward migration of OGZ with time.



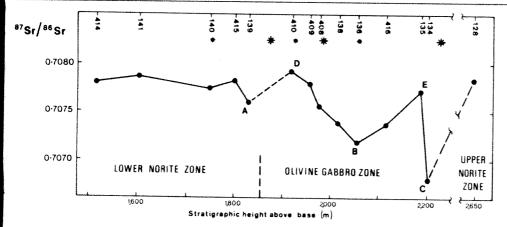


Fig. 2 Isotopic variation in the transition from the lower norite zone to the olivine gabbro zone, Kalka Intrusion. Sample numbers are shown along the top axis (major olivine component indicated by large asterisk, minor olivine component by small asterisk). Location of the section is shown in Fig. 1.

magma body were continuously mixed and isotopically homogeneous at any one time, the only explanation would involve addition from outside of material with a higher <sup>87</sup>Sr/<sup>86</sup>Sr ratio, either as direct contamination by wall rock or an influx of more contaminated magma. Accordingly, fluctuations in isotopic composition would reflect the interplay between the low and high <sup>87</sup>Sr/<sup>86</sup>Sr ratio sources; but while plausible, such a mechanism would probably produce random variations in contrast to the more regular observed pattern. Rather, the periodic return to a relatively constant isotopic ratio suggests that the new magma pulses were interacting with an almost infinite reservoir of contaminated magma; that they initially affected isolated aliquots of this reservoir, but were eventually absorbed by the reservoir. Increases in initial ratio, such as to D and E of Fig. 2, would then be due to a return to the background value of the intrusion.

The preferred model begins at the end of lower NZ time with the Kalka magma chamber filled by a well mixed contaminated basaltic liquid of relatively low density (<2.66)6 as a result of considerable crystallization of the pyroxene that accumulated in the pyroxenite zone; it is precipitating the two pyroxenes and plagioclase of the NZ with the high <sup>87</sup>Sr/<sup>86</sup>Sr ratio of 0.7078. A small pulse of primary magma enters the chamber and floods the floor due to its higher density. In moving across the floor the influx partly mixes with the adjacent resident magma forming a hybrid, which, still with a higher density than the main body of magma, forms an isolated layer that dominates the basal crystallization zone (evidence for basal crystallization in Kalka has been presented elsewhere<sup>2</sup>). The precipitated phases now reflect the composition of the hybrid: <sup>87</sup>Sr/<sup>86</sup>Sr ratios decrease and a short burst of olivine deposition as an olivine gabbro layer marks the beginning of crystal fractionation. The details of the petrological changes induced are determined by the nature of

the magma influxes. For example, sequential small pulses over a period of time will produce the gradual decline to minimum B (Fig. 2) whereas a single major pulse produces the abrupt drop to C.

The facies relationship between the OGZ and NZ is due to the restricted ponding of the magma pulses at low points on the floor of the intrusion: at any stage only part of the accumulative interface is disturbed and changes in the position of the low points with time can account for the eastern lateral migration of the main OGZ up-section (Fig. 1). The correspondence of the maximum lateral extent of the OGZ, the most magnesian olivine and the most calcic plagioclase, and the minimum initial <sup>7</sup>Sr/<sup>86</sup>Sr ratios in the upper parts of the OGZ are consistent with the greatest relative volumes of fresh magma injections being added to the system at that time. Ultimately, convectional mixing within the chamber as a whole disperses the residual hybrid, with crystallization reverting to the initial NZ state to await further magma pulses. At the same time, incorporation of the hybrid slightly lowers the background 87Sr/86Sr ratio of the main body of magma.

Independently, Huppert and Sparks<sup>7,8</sup> have proposed a similar model on fluid dynamical grounds thereby providing the physical basis of the processes described above. In particular, they demonstrate the formation of a stable basal layer by the incoming batch of magma and the eventual convective dispersal of the layer on cooling when density equilibration occurs. The link between petrological, fluid dynamic and isotopic approaches augurs well for the future elaboration of the evolution of layered intrusions and can give a more specific explanation of the origin of some types of macroscopic layering and cyclic units.

We conclude that the lithological transition from a monotonous gabbronorite sequence to a zone of vertically and

Table 1	Rb-Si	analytical	data for	Kalka	igneous rocks	•
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Sample	Lithology	Rb (p.p.m.)	Sr (p.p.m.)	<sup>87</sup> Rb/ <sup>86</sup> Sr	<sup>87</sup> Sr/ <sup>86</sup> Sr present	<sup>87</sup> Sr/ <sup>86</sup> Sr 1,150 My		
414	Melagabbronorite	1.17	73.1	0.0464	$0.70855 \pm 4$	0.70779		
141	Leuconorite	1.24	226.8	0.0158	$0.70811 \pm 6$	0.70785		
140	Olivine gabbronorite	1.10	133.7	0.0238	$0.70812 \pm 5$	0.70773		
415	Melagabbro	0.43	108.9	0.0115	$0.70801 \pm 6$	0.70782		
139	Gabbro	0.39	106.5	0.0107	$0.70777 \pm 4$	0.70759		
140	Olivine gabbro	0.59	102.7	0.0165	$0.70818 \pm 3$	0.70791		
409	Gabbronorite	0.87	116.8	0.0216	$0.70814 \pm 4$	0.70778		
408	Olivine melagabbro	0.73	63.8	0.0332	$0.70810 \pm 3$	0.70755		
138	Gabbronorite	0.88	141.1	0.0181	$0.70768 \pm 4$	0.70738		
136	Olivine gabbro	0.50	98.1	0.0146	$0.70742 \pm 4$	0.70718		
416	Melagabbro	0.61	82.2	0.0216	$0.70773 \pm 4$	0.70737		
135	Gabbronorite	0.58	114.2	0.0146	$0.70795 \pm 8$	0.70771		
134	Anorthosite	0.31	254.7	0.0035	$0.70683 \pm 6$	0.70679		
128	Gabbronorite	1.27	165.5	0.0222	$0.70821 \pm 6$	0.70784		

Sr isotopic ratios were measured at La Trobe University using a 22-cm radius solid-source mass spectrometer of modified Clement design. Uncertainties quoted in present-day  $^{87}$ Sr/ $^{86}$ Sr ratios are  $\pm 2\sigma$  mean. Measurements are relative to a value of  $0.71022\pm 4$  for the SRM 987 Sr standard. Initial ratios are calculated for an age of 1,150 Myr using  $\lambda$   $^{87}$ Rb  $1.42\times 10^{-11}$  yr $^{-1}$ . The age is an arbitrary mean for the 1,200–1,100 Myr time interval for emplacement of the Kalka Intrusion.

laterally restricted olivine gabbro horizons in Kalka correlates with the onset of cyclic fluctuations in initial 87Sr/86Sr ratios. Isotopic variability is attributed to dynamic mixing between the main body of contaminated magma in the chamber and incoming pulses of primary basaltic liquid. New magma batches ponded on the floor of the chamber temporarily dominating part of the basal crystallization zone; the result, transient olivine precipitation in laterally limited olivine gabbro layers having low Sr initial ratios. Ultimately, convective dispersal of the pools returned crystallization to its initial state with an accompanying rise in initial 87Sr/86Sr ratios to original or slightly reduced values

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# Shallowing-upward cycles in the Middle Proterozoic Altyn Formation

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Phanerozoic carbonate rocks commonly contain cycles, a few metres thick, which were deposited during progressive shallowing of their oceanic environment1. Each cycle represents a deepening of water in a nearshore area, followed by the accumulation of sediments up to sea level. The causes of the water deepening which begins each cycle are rarely known. Possibilities include tectonic activity that affected the depositional basin and changes in ocean volume due to the expansion and contraction of ice sheets. I report here that similar cycles occur in the Middle Proterozoic Altyn Formation, part of the Belt Supergroup<sup>2</sup>. The cycles are probably related to vertical tectonic activity which influenced sedimentation in the Belt basin. The presence of shallowing-upward cycles in the Altyn Formation shows that such cycles are not confined to the Phanerozoic. Other Precambrian sequences should be examined to see if they too contain cycles. Where such cycles are found they may give important information about Precambrian tectonism and glaciation.

The Altyn Formation is exposed in the eastern part of Glacier National Park, Montana. The age of the formation may be estimated from geochronological data available for equivalent stratigraphic units. The Aldridge Formation of British Columbia has granodiorite intrusives which are at least 1,335 Myr old<sup>3</sup> and a sill in the lower part of the Prichard Formation in Idaho is 1,430 Myr old (R. E. Zartman, unpublished data). A best estimate of the age of the Altyn Formation is  $1,450 \pm 50$  Myr (J. Harrison, personal communication).

I have divided the Altyn Formation into the informal members shown in Fig. 1. Seventeen cycles have been identified in member A with an average thickness of 4.2 m and a range of 1.6-8.0 m. Member C has 12 cycles ranging from 1.6 to 4.6 m thick with an average of 2.5 m. Members B and E are quite homogeneous and devoid of stromatolites or other palaeoenvironmentally diagnostic sedimentary structures, and so far no cycles have been recognized in them.

An idealized and simplified version of the Altyn Formation cycles is shown in Fig. 2. The cycles are bounded by erosion The lowest part of each cycle usually has conglomerates and breccias composed of stromatolite fragments and pebbles of quartz and microcline. Dolarenites composed of peloids and intraclasts with variable amounts of quartz and microcline overlie the coarse basal layer. These dolarenites occasionally contain tabular cross-bedding. In the upper part very similar dolarenites alternate with stratiform stromatolites. The dolarenites commonly contain lenticular bedding and bimodal cross-bedding produced by the alternating flow of tidal currents. The stromatolites are very similar to those formed by smooth algal mats in modern lower intertidal zones of Shark Bay<sup>4,5</sup> and the Persian Gulf<sup>6</sup>. Smooth mat development is inhibited in Shark Bay and the Persian Gulf in areas where sand substrates are kept moving by strong tidal currents and waves<sup>5,6</sup>. Similarly the stratiform stromatolites of the Altyn Formation formed during relatively low energy conditions in the intertidal zone. This development of stromatolites was interrupted periodically by higher energy events, such as storms, which brought in sands from the adjacent subtidal zone. There is usually an upward increase in the ratio of stromatolites to dolarenites in each cycle, showing the decreasing influence of sand input from subtidal areas. Many stromatolites in the upper part of cycles have desiccation cracks and their upper surfaces are broken and disrupted to form intraformational breccias. These features are the result of increasing subaerial emergence and consequent drying of the sediments during the development of the upper part of the cycles. Each cycle represents deposition in progressively shallower water as sediments built up to sea level following an increase in water depth. Subtidal sands are overlain by lower intertidal sands and stromatolites which in turn pass upward into upper intertidal and supratidal stromatolites and intraformational breccias and conglomerates. This sequence is typical of cycles found in many Phanerozoic carbonate rocks1. There are few reported examples of shallowingupward cycles from rocks of Precambrian age. One possibility is the Strelley Pool chert which occurs in the 3,400-Myr old Warrawoona Group of Western Australia. This is an evaporitecarbonate sequence containing stromatolites. The Strelley Pool chert was deposited in shallow subtidal to intertidal environments<sup>7</sup>, and may represent an overall shallowing-upward sequence.

There are two important modifications of the idealized shallowing-upward cycle described above. Member A frequently contains discontinuous bioherms of columnar stromatolites near the base of the stromatolite-rich part of cycles. These commonly occur with conglomerates and breccias and overlie erosion surfaces. The columnar stromatolites were formed in localized higher energy environments in the lower intertidal zone, especially in tidal channels8. Many member C cycles contain arkoses

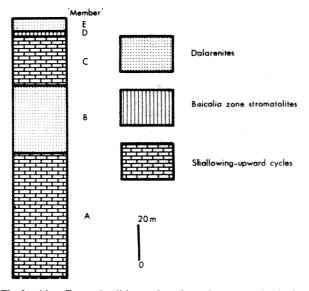


Fig. 1 Altyn Formation lithostratigraphy at the type section in the Appekunny Creek area, Glacier National Park, Montana

and shales, mainly in their upper parts. These terrigenous sediments were deposited in the upper intertidal and supratidal zones during flash flooding from the adjacent land area.

Further support for shallowing-upward depositional conditions in Altyn Formation cycles is provided by the former presence of evaporite minerals and the dolomitization of carbonate minerals. The evidence for evaporites includes silica pseudomorphs after gypsum crystals and anhydrite nodules, often with micrometre-sized gypsum and anhydrite preserved in the silica. The pseudomorphs contain length-slow chalcedony and flamboyant quartz, both of which replace evaporite minerals9,10

Cellular vugs filled by a centripetal sequence of silica, dolomite and calcite also occur. Some of these have a 'chickenwire' fabric which is characteristic of many modern and ancient anhydrite nodules 10. The formation of displacive gypsum crystals and anhydrite nodules is a diagenetic process which takes place in sediments as a result of intense evaporation of inter-stitial water in arid climates<sup>1,11</sup>. Gypsum crystals grow within sediments in the upper intertidal zone and anhydrite forms in the supratidal zone<sup>11</sup>. Sediments deposited in shallow subtidal or lower intertidal zones may be exposed later to upper intertidal or supratidal zone conditions as a result of progradation. Evaporites may form in these sediments because of this change of environment. The precipitation of calcium sulphate evaporite minerals in these sediments produces magnesium-rich interstitial waters which may lead to dolomitization of carbonate minerals<sup>1,11</sup>. In the Altyn Formation some columnar stromatolites and lower intertidal sediments contain replaced evaporites, showing that they were subsequently exposed to upper intertidal and supratidal conditions. All of the original carbonate minerals in each of the cycles are dolomitized as a result of these changes of environment. The dolomitization and formation of evaporites could have occurred only in shallowingupward cycles following sediment aggradation and the resultant seaward shift of the tidal zones.

Detailed studies show that many Phanerozoic carbonate rocks are cyclic and that each cycle represents aggradation during progradation, following a rapid transgression during which little sediment was deposited 1,12,13. The accumulation of a sequence of shallowing-upward cycles requires repeated transgressions, the cause of which is not well understood. Some possibilities include differential subsidence due to tectonic activity, eustatic sea level changes resulting from glaciation or changes in ocean volume caused by global tectonics<sup>14</sup>. The period 2,300-950 Myr ago was characterized by warm climates, with no evidence of glacial periods<sup>15</sup>. Thus glacial eustasy seems not to be the cause of cyclic sedimentation in the Altyn Formation. Opinions differ regarding the tectonic setting of the Belt depositional basin. One

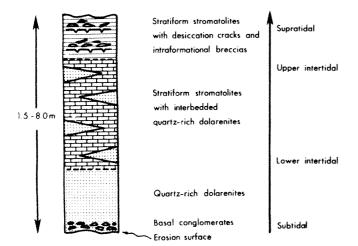


Fig. 2 Simplified shallowing-upward cycle representative of those found in the Altyn Formation.

view is that the basin was located at a rifted continental margin of the passive or Atlantic type 16-18, with vertical tectonic activity during deposition18. An opposing view suggests that the Belt basin was one of several, possibly fault-bounded, basins located within a continental craton 19,20.

Major contemporaneous faulting at the southern margin of the Belt basin strongly influenced sedimentation of the coarsegrained LaHood Formation<sup>21</sup>. This formation is equivalent in age to the Altyn Formation. Thus cyclic sedimentation in the Altyn Formation was probably related to intermittent subsidence caused by vertical tectonic activity, with sediment accumulation occurring in quieter periods between the tectonic events. Sedimentation elsewhere in the Belt basin was probably affected by tectonic activity. Detailed studies of other stratigraphic units of the Belt Supergroup may reveal cycles or manifestations of this tectonism. The presence of small-scale cycles in the Altyn Formation suggests that Precambrian sequences in other localities should be examined closely to see if they also contain cycles.

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# Superoxide dismutase is a prophylactic against alloxan diabetes

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A single injection of alloxan<sup>1</sup>, or alloxan-like derivatives of uric acid<sup>2,3</sup>, kills the insulin-producing islet  $\beta$ -cells and causes diabetes mellitus in animals. This effect is probably mediated by a sequence of redox reactions involving the production of superoxide anion radicals in or near the  $\beta$ -cells<sup>4-8</sup>. Superoxide anions may also arise in inflammation 9-15, and islet inflammation often accompanies the outbreak of human diabetes16, perhaps following the combined onslaught of viruses and chemical agents on the  $\beta$ -cells<sup>17</sup>. We now report that injections of superoxide dismutase, an enzyme removing superoxide anion radicals, act prophylactically against alloxan-induced diabetes.

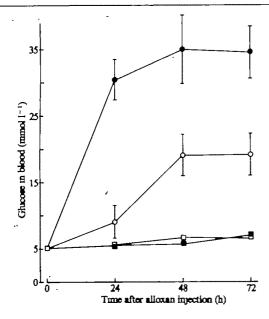


Fig. 1 Blood glucose concentrations in uninjected mice (0 h) and in mice intravenously injected with 25 (III), 50 (O) or 75 (Φ) mg alloxan per kg body weight Alloxan monohydrate (U S. Biochemical Corporation) was dissolved in a salt-balanced Krebs-Ringer buffer that had been adjusted to pH 4.0 by the addition of HCl. Female 3-month-old non-inbred mice (21-26 g) were mjected into a tail vem with alloxan solution at a dozage of 100 μl per 20 g mouse; control mice were similarly injected with physiological saline (□). Blood samples (25 μ leach) were taken from the cut tail tips 24, 48 and 72 h after injection, precipitated by ZnSO<sub>4</sub> and NaOH and assayed by an automatic glucose oxidase/peroxidase technique (Glox reagents, Kabl). Each point is the mean for five different mice; vertical bars show a.e. m. when larger than the symbol indicating the mean value.

When mice were injected into a tail vein with alloxan (50 or 75 mg per kg body weight), they became markedly hyperglycaemic within 48 h (Fig. 1). The diabetogenic effect was clearly dose-dependent, as alloxan at 25 mg per kg had no effect and 50 mg per kg produced a milder hyperglycaemia than did 75 mg per kg. With increasing concentrations, alloxan attacks cells other than the islet  $\beta$ -cells, for example, kidney and liver cells. The unequivocal, though not maximal, diabetogenic action of 50 mg per kg would provide optimum conditions for detecting any protecting or potentiating effect of the substances to be tested, and so this intermediate alloxan dose was selected for the subsequent experiments.

As shown in Table 1, in each of two separate series of experiments the diabetogenic action of alloxan was largely prevented if the mice had been injected with superoxide dismutase 12 h earlier. The enzyme preparation injected (provided by K.-E. Arfors, Pharmacia) was a purified yeast CuZnsuperoxide dismutase covalently linked to polyethylene glycol chains to yield a complex of molecular weight 100,000, so as to slow down its elimination from the circulation 16. Whereas the circulating half life of uncoupled superoxide dismutase in the rat is only 6 min (ref. 14), the enzyme-polyethylene glycol complex had an initial half life of about 2 h (Fig. 2). The elimination was more complicated than a first-order process, and after 12 h as much as 6% of the initial activity remained in the circulation. This remaining activity was about 10 times the basal activity of superoxide dismutase measured in the plasma of four untreated control mice  $(505\pm19 \text{ U ml}^{-1}; \text{ mean}\pm\text{s.e.m.})$ .

The antidiabetogenic action of superoxide dismutase was specific, as control experiments revealed no effect of inactivated superoxide dismutase—polyethylene glycol or of polyethylene glycol alone (Table 1). Furthermore, the enzymatically active superoxide dismutase—polyethylene glycol had no effect on blood glucose levels in healthy mice not treated with alloxan (Table 1).

According to the free-radical hypothesis of alloxan diabetogenicity, reduction of alloxan to dialuric acid in the islets is followed by autoxidation back to alloxan accompanied by the formation of superoxide anion radicals and hydrogen peroxide  $^{4-8}$ . Highly reactive hydroxyl radicals are then formed by a metal-catalysed 'Haber-Weiss reaction' that can be seen as the summation of the reduction of ferric ion by superoxide (Fe<sup>3+</sup> + O<sub>2</sub>.  $\rightarrow$  Fe<sup>2+</sup> + O<sub>2</sub>) and the Fenton reaction (H<sub>2</sub>O<sub>2</sub>+Fe<sup>2+</sup>  $\rightarrow$  OH·+OH<sup>-</sup>+Fe<sup>3+</sup>). The extreme and indiscriminatory reactivity of hydroxyl radicals can explain why alloxan rapidly affects a wide range of phenomena in the  $\beta$ -cells, including insulin synthesis<sup>19</sup>, insulin release<sup>8,20</sup>, cation pumping<sup>6,21</sup>, glucose metabolism and oxygen consumption<sup>22</sup>, Trypan blue exclusion<sup>6,23</sup> and mitochrondrial morphology<sup>24</sup>. In support of this hypothesis alloxan toxicity is counteracted *in vivo* by scavengers of hydroxyl radicals and *in vitro* by such scavengers<sup>6,23</sup> as well as by superoxide dismutase, catalase and a chelator of iron ions<sup>6-8</sup>. The present results represent further, and crucial, support for the hypothesis, as they confirm the

Table 1 Prophylaxis by superoxide dismutase against alloxan diabetes in mice

Drug injected before	Time between injections of SOD/PEG (or PEG) and alloxan		ose (mmol I <sup>-1</sup> ) NaCi
First experimental series			
None	_	$20.4 \pm 2.4 (5)$	4.8 + 0.1(9)
SOD-PEG, 200 mg per kg	· 12 h	$9.2 \pm 1.4^{*}$ (6)	$50\pm02(4)$
Inactivated SOD-PEG 200 mg per kg	, 12 h	$21.1 \pm 2.5 (5)$	$5.2 \pm 0.2 (5)$
Second experimental series			
None		$21.4 \pm 2.4$ (6)	$4.8 \pm 0.3(4)$
SOD-PEG, 250 mg per kg	12 h	$8.2 \pm 2.1*(6)$	$5.7 \pm 0.1 (6)$
Inactivated SOD-PEG 250 mg per kg	, 12 h	20.4±1.9 (6)	$5.4 \pm 0.2 (6)$
PEG, 250 mg per kg	12 h	$19.0 \pm 1.1 (5)$	$5.6 \pm 0.3 (5)$
PEG, 250 mg per kg	2 h	$17.9 \pm 1.2$ (5)	$5.1 \pm 0.3$ (5)

Mace were injected with alloxan (50 mg per kg) or NaCl, and blood glucose was assayed after 72 h as in Fig. 1. Before injection with alloxan or NaCl, groups of mice were injected into a tail vein with yeast CuZn-superoxide dismutase coupled to polyethylene glycol (SOD-PEG), with SOD-PEG that had lost 99.2% of its enzyme activity during prior exposure to  $\rm H_2O_2$  (ref. 6), or with uncoupled polyethylene glycol alone (PEG). The time elapsing between the injection of SOD-PEG or PEG on the one hand and alloxan or NaCl on the other was 12 or 2 h as indicated. All mice were firsted, with free access to water for 12 h before the alloxan or NaCl injection and for 3 h before the blood sampling. Results are given as mean values  $\pm$ s.e.m. for the numbers of animals stated in parentheses. In the two separate series, different batches of SOD-PEG were used.

two separate series, different batches of SOD-PEG were used.

\* The prophylactic effect of SOD-PEG against alloxan diabetes was against and, with P < 0.01 in either series of experiments (Student's *t*-test, two-tailed). Among the 11 mice injected with alloxan alone, the lowest and highest blood glucose values were 11.2 and 28.1 mmol  $1^{-1}$ . The corresponding range for the 13 mice injected with NaCl alone was 3.9–5.5 mmol  $1^{-1}$ . Among the 12 mice injected with alloxan after 200 or 250 mg per kg SOD-PEG, as many as 6 had blood glucose values in the range of 4.7–5.9; the remaining 6 values ranged over 8.5–17.0

postulated role of superoxide anion radicals in the diabetogenic action of alloxan *in vivo*. Even more important, apparently for the first time it has been possible to protect against alloxan diabetes by administering a drug several hours beforehand. This result may be of more than theoretical interest, as superoxide dismutase has already been established as a safe drug of potential value for the treatment of other diseases in humans<sup>26</sup>.

It has been suggested that the superoxide anion radical is relatively harmless in  $vivo^{27.28}$ , based on the relatively low reactivity of the radical with several classes of compound in vitro, and the assumption that other reductants might substitute for it in reducing the ferric ion in the Haber-Weiss reaction. Against this background our results seem important in demonstrating the high toxic potential of the superoxide radical in vivo, as well as in underlining the importance of an adequate protection against it. The results do not necessarily mean that  $\beta$ -cells are

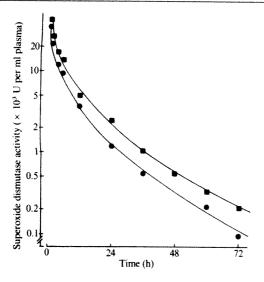


Fig. 2 Superoxide dismutase activity in the blood plasma of two mice at various times after an intravenous injection of yeast CuZn-superoxide dismutase coupled to polyethylene glycol; the drug was injected into a tail vein at a dosage of 200 mg per body weight. Samples (25 µl each) of whole blood were drawn from the tails and mixed with  $500\,\mu l$  of a solution containing  $150\,mmol\,l^{-1}$  NaCl and  $3\,mmol\,l^{-1}$  EDTA. After centrifugation, the supernatant was assayed for superoxide dismutase by the direct spectrophotometric method using KO<sub>2</sub> (ref. 29). One unit of enzyme activity corresponds to 4.2 ng of bovine CuZn-superoxide dismutase. Plasma levels were calculated by assuming an erythrocyte blood volume fraction of 40%. To show the elevation of enzyme activity produced by injecting SOD-PEG, the values in the figure have been corrected for the mean endogenous superoxide dismutase activity in mouse plasma (505 U ml<sup>-1</sup>).

especially sensitive to alloxan because they are extraordinarily sensitive to the harmful effects of superoxide radicals. It seems equally possible that the physiological specialization of the  $\beta$ -cells renders them unusually effective in reducing alloxan or in catalysing the iron-dependent formation of OH. from H<sub>2</sub>O<sub>2</sub> and O2.

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# Nepotism among rhesus monkey brothers

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Either during or shortly after sexual maturation, male rhesus monkeys leave the social groups in which they were born and join other non-natal groups (refs 1, 2 and unpublished data). Here we report that males frequently transfer into the same social groups as their older maternally related brothers. Such brothers spent more time close to each other than to other males, formed alliances with brothers more frequently than with non-brothers and disrupted each other's interactions with oestrous females less frequently than expected by chance. They also spent more time in non-natal groups than did males who did not have a brother in the group. Length of time in a group is positively associated with male dominance rank<sup>1</sup> and probably with reproductive success. The data are consistent with proposals that brothers who behave nepotistically and noncompetitively have high inclusive fitness.

Data are presented from long-term census records and 339 h of focal-animal<sup>3</sup> observations (February 1978–February 1979) of 18 male rhesus monkeys who comprised seven sets of siblings: a quintet, a triad and five pairs. The subjects lived in four social groups containing ~200, 175, 160 and 110 animals (groups A, I, C and E, respectively). The four study groups and four other social groups were free-ranging on La Cueva, a provisioned 100-acre island off the south-west coast of Puerto Rico<sup>4</sup>. Three of the four study groups were introduced to the island in 1962, the fourth in 1969 and four smaller groups in 1975. One other group was introduced in 1962, but decreased in size and disintegrated by 1975. Each monkey had an identification tattoo of letters and numbers and the maternal relations were known for all animals in the four study groups4.

During 20-min observation periods, the frequency and duration of approaches within 2 m (excluding agonistic encounters) between the focal male and all other individuals in his group were recorded, as were all his involvements in agonistic encounters and his interactions with oestrous females. All but three of the subjects were observed for 51 periods. Two subjects died (G6 and N6, 21 and 33 observations, respectively) and one (CT5, 30 observations) was observed from mid-study. By comparing each subject's interactions with brothers and nonbrothers during only his set of observations, no bias is introduced by unequal samples of observations. For all subjects, equal numbers of observations were made during each of two time periods: 06.30-09.00 and 09.00-11.30 h.

Analysis of group transfer among young males suggested that they shifted into groups containing older brothers. Although males transfer preferentially into the non-natal group with the highest ratio of adult females to adult males<sup>1</sup>, less than half of the males that have ever joined a brother's group on La Cueva (n = 17) joined the non-natal group with the highest ratio. Each social group on La Cueva often came into contact with all other groups5 and there was no evidence that geographical location of groups influenced group transfer. In addition, no male who was transferring from his natal group had a brother in more than one non-natal group.

If we assume the distribution of males in non-natal groups to be random, the probability of a male's joining his brother(s) in

one of three study groups was 0.33. This expected probability was an overestimate for some males, because after 1975 there were four small groups that they could have joined (which did not contain older brothers of any transferring males). By assuming a larger probability, however, it is more difficult to reject the null hypothesis that group transfer is independent of kinship. Since 1962, there have been 31 males who had a brother in a non-natal group when they changed groups. Seventeen joined their brothers' groups while 14 did not ( $\chi^2 = 6.5$ , d.f. = 1, P <0.05). At the end of the study, 9 of 14 living males who had left their natal group and had an older brother in a non-natal group were in their older brothers' groups ( $\chi^2 = 6.2$ , d.f. = 1, P <0.05). Males from the same natal groups were not significantly clumped ( $\chi^2 = 13.69$ , d.f. = 11, P > 0.10).

Fourteen of the 18 subjects approached their brother(s) more frequently than they approached any other males (P < 0.05, sign test), and brothers spent more time within 2 m of each other  $(\bar{x} = 24.5 \text{ min})$  than of any other males  $(\bar{x} = 7.7 \text{ min}, P < 0.05,$ Mann-Whitney U). During focal observations 19 coalitions were formed between a subject and another male during a fight with a third male (Table 1). Of the 10 different subjects involved in a coalition, 9 more frequently aided a brother than a nonbrother (P < 0.05, sign test). Agonistic coalitions have been reported for several macaque species<sup>6-8</sup> and probably have a significant effect on the success of brothers in non-natal groups. This hypothesis is supported by the dominance rank history of the sibling triad G6, N6 and 8G. The oldest brother disappeared during a food shortage and several weeks later the middle brother (N6) was attacked and died from infection. The youngest brother (8G) immediately dropped four positions in the male dominance hierarchy, the only male to decrease more than one rank-position during the study.

When an oestrous female was soliciting or consorting with a male, she was often displaced from that male (or attacked) by another adult male (n = 156 disruptions, Table 2). For 13 of 15 subjects, the observed number of disruptions involving brothers was less than the expected value based on males randomly receiving or sending disruptions (P < 0.05, sign test). These data suggest that brothers avoided disrupting each other's sexual interactions.

During the colony's history, brothers who had been in the same group (n = 26) accrued more months in those groups  $(\bar{x} = 31.7)$  than did the 25 males who were not in their brothers groups ( $\bar{x} = 21.4$ , P < 0.05, Mann-Whitney U). This difference was not due to age, as the males who did not join their brothers' groups were older  $(\bar{x} = 8.7 \text{ yr})$  than those who did join their brothers ( $\bar{x} = 8.1 \text{ yr}$ ). Nor was the difference due to the maternally dependent ranks of the males9. Those from the topranking half of their natal group did not spend significantly more time in non-natal groups than those from the bottom-ranking half ( $\bar{x} = 26.2$  and 27.9 months, respectively).

As a male accrues time within a non-natal group, he increases not only the number of breeding seasons during which he can

Table 1 Numbers of coalitions observed between the subjects and their brothers or non-brothers

Coalition between:						
Subject	Brothers	Non-brothers	Sign			
91	1	0	+			
E4	1	0	+			
1 <b>D</b>	1	0	+			
7Z	3	0	+			
CV7	3	1	+			
AC9	1	0	+			
CT5	2	0	+			
1L	0	1	*****			
7S	2	0	+			
G6	1	0	+			

Nine of the 10 subjects were more frequently involved in a coalition with a brother than with a non-brother (P < 0.05, sign test).

Table 2 Disruptions of interactions with cestrous females involving brothers

**************************************				aptions involving brother		
	Total		Expected	Observed		
Subject	disruptions	P	(E)	(O)	Sign	
91	13	0.071	0.94	0		
5C	8	0.071	0.57	0	venim	
E4	8	0.308	2.46	1	Person	
O8	5	0.308	1.54	1	rinky	
1D	9	0.308	2.77	2	~~~	
7P	19	0.308	5.85	5		
2T	10	0.308	3.08	3	****	
7Z	6	0.091	0.55	1	+	
CV7	2	0.091	0.18	0	September	
AC9	9	0.091	0.82	0		
CT5	7	0.091	0.64	1	+	
1L	12	0.067	0.80	0	- Colonia	
7S	21	0.067	1.41	1	and the	
$\mathbf{X}0$	12	0.067	0.80	0	- Charles	
2L	15	0.067	1.01	0	-	

Probability (P) of a brother sending or receiving a disruption = (no. ofbrothers subject has in group) ÷ (no. of reproductively active males in group -1). Expected frequency of a disruption involving a brother equals the product of P and the total number of disruptions. Sign decision is based on whether observed brother disruptions are greater or less than expected number of brother disruptions (P < 0.05, sign test).

father offspring, but also his dominance rank<sup>1,10</sup>. As there is a positive association between dominance rank and RS11,12, a male who accrues time (and therefore rank) in a group probably sires, on the average, relatively more offspring each breeding season (ref. 10 and unpublished data).

Affiliations among brothers have been described briefly for several nonhuman primates<sup>13,14</sup>. One possible mechanism by which brothers develop affiliative behaviours is attachment<sup>15</sup> which may result from their close association with their mother. This hypothesis is supported by the fact that the average age difference between brothers in the same groups was 1.7 yr, while that between brothers in different groups was 2.6 yr. Other data, however, suggest that monkeys raised in isolation from birth associate preferentially with unfamiliar siblings<sup>16</sup>. Hence, both kin recognition and attachment may result in nepotism. Whatever the mechanisms which result in affiliative behaviours among brothers, our data are consistent with the hypothesis that nepotistic brothers have high inclusive fitness, and Hamilton's model<sup>17</sup> remains a plausible explanation for the evolution of nepotism among rhesus monkey brothers.

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# Opiates and clonidine prolong calcium-dependent after-hyperpolarizations

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Opiates<sup>1,2</sup> and  $\alpha$ -agonists<sup>3,4</sup> bind to separate and structurally specific sites on neurones in the central<sup>1,3</sup> and enteric nervous systems<sup>2,4</sup>. One functional consequence of this binding is inhibition of cell firing<sup>5,6</sup>, which may be due to hyperpolarization of the resting membrane by opiates, such as has been observed in the guinea pig locus coeruleus<sup>7</sup> and myenteric plexus<sup>8</sup>, and by clonidine in the myenteric plexus9. However, the discharge frequency of many nerve cells is limited by the membrane hyperpolarization which follows a period of activity, and which is caused by a transient increase in the intracellular calcium concentration leading to the activation of a membrane potassium conductance<sup>10</sup>. Neurones of the guinea pig myenteric plexus exhibit such a calcium-dependent potassium conductance11-13. We now report that both opiates and clonidine prolong this calcium-dependent after-hyperpolarization at concentrations (100 pM-10 nM) which are considerably lower than those usually required to hyperpolarize the resting membrane. Such a prolongation of the after-hyperpolarization will limit the frequency of discharge of neurones without altering their resting potential. The nature of the effects of morphine and clonidine are of interest in view of the similarities between the anatomical distribution of binding sites for these two drugs and the close parallels between their pharmacological effects in animals and man.

Intracellular recordings were made for more than 60 neurones in myenteric ganglia isolated from guinea pigs<sup>12</sup>. The neurones were bathed in a physiological saline solution (see Fig. 1 legend) to which morphine, clonidine or other drugs could be added. Action potentials were evoked by passing brief depolarizing currents through the recording electrode. Most of the experiments were on neurones which showed long-lasting after-hyperpolarization following a single action potential (type 2 (ref. 12) or AH cells<sup>13</sup>). Type 1 cells<sup>12</sup> or S cells<sup>13</sup> only showed an after-hyperpolarization following a train of action potentials. The after-hyperpolarization following a train of action potentials (usually 10 Hz for 3 s) was completely eliminated by cobalt (3 mM) or by perfusion with calcium-free solution (Fig. 1).

Morphine (100 pM-10 nM) prolonged the after-hyper-polarization which followed a train of action potentials. The effect of morphine was concentration dependent in the range tested, and reversed within 5-10 min of washing with morphine-free solution (Fig. 2). Naloxone (1 nM) reversibly abolished the action of morphine. The prolongation occurred without marked change in amplitude or time course of the initial component of the after-hyperpolarization (see Fig. 2 legend), suggesting that morphine may inhibit the mechanism by which a neurone normalizes its free intracellular calcium concentration following the action potentials.

Doubling the extracellular calcium concentration or the application of tetraethylammonium bromide (1 mM), increased the duration of the after-hyperpolarization. Morphine was much less effective in further prolonging the after-hyperpolarization

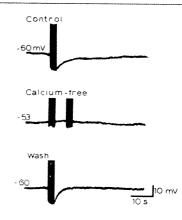


Fig. 1 Abolition of the hyperpolarization following a train of action potentials by removal of extracellular calcium ions. This and other figures are intracellular recordings of membrane potential. Depolarizing current pulses of sufficient amplitude to evoke action potentials were passed across the cell membrane (full spike amplitude is not shown). Each train consisted of 15 pulses at 5 Hz. Top trace, control recording. Resting potential, -60 mV. Middle trace, 20 min after changing to a solution containing no calcium and 5 mM magnesium. The cell membrane depolarized by 7 mV, but even two trains of action potentials failed to elicit any after-hyperpolarization. Bottom trace, 20 min after returning to the control solution. All intracellular recordings were done using techniques described elsewhere 12. Control perfusion solutions contained (mM): NaCl, 117; KCl, 4.7; CaCl<sub>2</sub>, 2.54; MgCl, 1.2; NaH<sub>2</sub>PO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25; glucose, 11, and were gassed with 5% CO<sub>2</sub> and 95% O<sub>2</sub>.

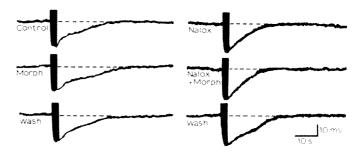


Fig. 2 Prolongation of the after-hyperpolarization by morphine (1 nM). Left: top trace, control recording; middle trace, 2 min after commencing perfusion with a solution containing morphine sulphate (1 nM); bottom trace, 10 min after washing with a morphine-free solution. Right: top trace obtained after several minutes in naloxone hydrochloride (1 nM); middle trace, 2 min after changing to a solution which contained naloxone and morphine (1 nM); bottom trace, 10 min after washing with control solution. Morphine had no effect in the presence of naloxone; in this and other experiments, naloxone itself slightly reduced the after-hyperpolarization. Maximum amplitude of after-hyperpolarization was not affected by morphine (1 nM); control, 17.5 ± 0.70 mV; morphine,  $18.4 \pm 0.53 \text{ mV}$  (mean  $\pm$  s.e., paired *t*-test, P > 0.05, n = 11). Time for decay to 50% ( $t_{50}$ ) or to 80% ( $t_{80}$ ) of peak amplitude was prolonged by morphine (1 nM): control,  $t_{50}$  = 12.2 ± 1.5 s; morphine,  $t_{50} = 16.0 \pm 2.0$  s (paired t-test, P < 0.01, n = 11). Control,  $t_{80} = 24.5 \pm 3.0$  s; morphine,  $t_{80} = 36.0 \pm 4.3$  s (paired t-test, P < 0.001, n = 11).

in the presence of an elevated (5.08 mM) calcium ion concentration (Fig. 3). Reducing the calcium ion concentration to one-half (1.26 mM) its control value decreased the duration of the after-hyperpolarization; in this case the effect of morphine became greater (Fig. 3). The lower calcium concentration may be much closer to that of 'free' calcium in the extracellular space of the nervous system<sup>14</sup>.

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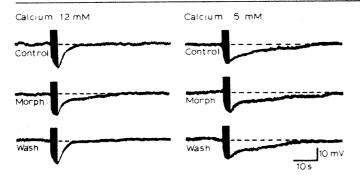


Fig. 3 Effect of morphine in high and low calcium concentrations. Left, recordings from the same neurone shown in Fig. 2, but after 30 min in a solution containing low (1.2 mM) calcium concentration. Top trace, control; middle trace, after 2 min perfusion with morphine (1 nM); bottom trace, wash. Right, further recordings from the same neurone, after 30 min in high (5 mM) calcium solution. Top trace, control; middle trace, after 1.5 min perfusion with morphine (1 nM); bottom trace, wash. The prolongation of the after-hyperpolarization by morphine was much more apparent when the after-hyperpolarization was shortened by reducing the calcium concentration.

Opiates inhibit the binding of calcium to brain synaptosomal membranes and the extent of this inhibition is reduced in high calcium solutions<sup>15</sup>. We speculate that occupation of the opiate receptor of myenteric neurones may lead to an inhibition of the binding of calcium to an intracellular site, perhaps on the inner surface of the membrane. If this site is normally important in the sequestration of calcium which enters the neurone during activity, such an inhibition would prolong the period of increased intracellular calcium and, therefore, the after-hyperpolarization. Such a mechanism could also underlie the hyperpolarization of the resting membrane observed with higher morphine concentrations8.

Clonidine (100 pM-10 nM) prolonged the after-hyperpolarization in a similar manner to morphine, although a larger proportion of neurones also showed a hyperpolarization of the resting membrane. This action of clonidine was prevented by phentolamine (100 nM). The similarity between the acute effects of clonidine and morphine may underlie the closeness of the pharmacological profiles of the two drugs. It is further possible that their identical mechanism of action offers an explanation for the efficacy of clonidine in ameliorating the signs and symptoms of opiate withdrawal<sup>6,1</sup>

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# Calmodulin localization during capping and receptor-mediated endocytosis

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The early response by lymphocytes to challenge with cell surface-directed ligand, such as antibody or concanavalin A (Con A), involves the clustering of initially diffuse ligandreceptor complexes into patches, followed by the gathering of these patches into a cap over one region of the cell and their endocytosis1-3. Using cells of the human lymphoblastoid line WiL2, we have previously shown that receptor-mediated endocytosis occurring at caps involves clathrin-coated vesicles and that the formation of such vesicles is sensitive to drugs such as trifluoperazine dihydrochloride (TFP) that affect calmodulin (CaM), the calcium-dependent regulatory protein of cells<sup>3</sup>. By indirect immunofluorescence, we demonstrate here that in WiL2, before surface-directed ligand challenge, CaM is distributed diffusely in the cell. On challenge, concurrent with capping of cell-surface receptors for Con A, CaM localization becomes concentrated in regions of the cytoplasm just below the cap. We show that CaM redistribution is sensitive to TFP and dependent on Ca2+ in the external milieu. Capping is mostly unaffected by TFP or removal of external Ca2+. Cytochalasin D (CD), on the other hand, blocks capping completely, but does not prevent the initial redistribution of CaM. These results are consistent with a functional role for CaM in clathrin recruitment to the cell surface beneath ligand-receptor complexes and an initial Ca2+ requiring, microfilament-independent event in receptor-mediated endocytosis.

Calmodulin has been implicated in the mediation of various cellular activities thought to involve transient fluxes of Ca<sup>2-</sup> signals<sup>4,5</sup>. In particular, CaM-Ca<sup>2+</sup> has been shown to regulate myosin light chain kinase of smooth muscle and non-muscle cells<sup>6-10</sup> and may be an important intermediate in the Ca<sup>2</sup> control of microfilament-based motility in such cells. Immunofluorescence techniques have been used to visualize the localization of CaM in a variety of cells 11-17. To study the intracellular distribution of CaM in WiL2 cells during Con A capping and endocytosis, we prepared rabbit antiserum against purified bovine brain CaM (Fig. 1a, b) as outlined by Wallace and Cheung<sup>18</sup>. This antiserum specifically identifies one polypeptide band, CaM, in a crude brain extract by a gel transfer peroxidase-antiperoxidase staining technique 19 (see Fig. 1). The antiserum has also been used in preliminary experiments specifically to immunoprecipitate WiL2 CaM metabolically labelled with <sup>35</sup>S-methionine (J. L. Salisbury and R. Green, unpublished observations). We further purified the anti-CaM IgG by affinity chromatography on a CaM-Sepharose column according to Dedman and co-workers<sup>11</sup> (see Fig. 1 legend) and, using the purified immunoglobulin as the primary antibody, we followed the reorganization of intracellular CaM during Con A receptor capping using double labelling techniques (Fig. 2). In these studies Con A receptors and CaM could be followed in the same cell using Con A covalently labelled with fluorescein isothiocyanate (FITC-Con A), which fluoresces yellow-green, and anti-CaM labelled with a secondary antibody, rhodamine-conjugated goat anti-rabbit IgG, which fluoresces red.

CaM localization during capping appears concentrated at sites of endocytosis. When WiL2 cells are fixed immediately after ligand challenge and labelled with anti-CaM (as described in Fig. 2g-i legend) they exhibit a diffuse cell-surface FITC-Con A localization (Fig. 2h) and a diffuse intracellular distribution of rhodamine-labelled anti-CaM (Fig. 2i) which is brighter than the diffuse low level of rhodamine fluorescence displayed by

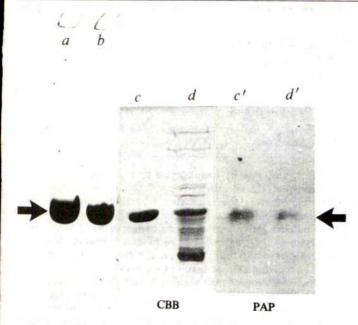
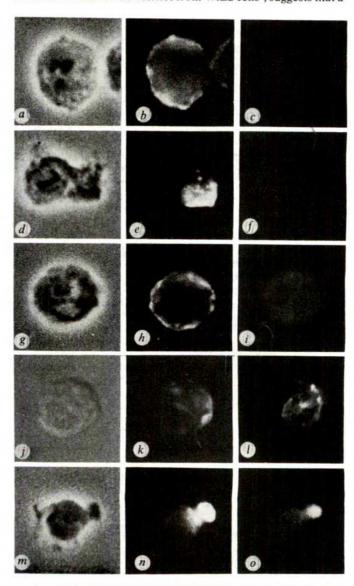


Fig. 1 Rabbit antiserum against bovine brain CaM was prepared according to Wallace and Cheung<sup>18</sup>. Track a is a SDS-polyacrylamide electropherogram showing a sample of the highly purified bovine brain CaM used to prepare the dinitrophenyl (DNP)–CaM shown in track b, which was subsequently used as the antigen. The antisera contained non-precipitating anti-CaM antibodies which were detected by the peroxidase–antiperoxidase staining of anti-CaM serum-treated nitrocellulose transfers from 15% polyacrylamide microslab gels containing 0.1% SDS into which either purified (tracks c, c') or heat-treated crude CaM containing extracts from bovine brain (tracks d, d') were electriphoresed. Shown here is a Coomassie brilliant blue stained gel (CBB) and a peroxidase–antiperoxidase (PAP) stained transfer of a companion gel. CaM is the band near the centre of each track (arrows). Specific anti-CaM antibody was affinity purified from the IgG fraction of immunized rabbit antiserum by adsorption on to native CaM covalently linked to Sepharose 4B. Following an extensive wash, specifically bound anti-CaM IgG was eluted with a pulse of 0.2 M glycine pH 2.8, adjusted to pH 7.5 with 1 M phosphate buffer and dialysed against phosphate-buffered saline (PBS).

Fig. 2 Indirect immunofluorescent localization of CaM during Con A receptor capping. Indirect immunofluorescent localization of anti-CaM binding sites was carried out using the affinity purified anti-CaM and rhodamine-conjugated goat anti-rabbit IgG (Cappel Laboratories). WiL2 cells were challenged with FITC-Con A (10 µg ml-1) to induce capping of Con A receptors. At various times after FITC-Con A challenge the cells were briefly fixed with formaldehyde, extracted in -20 °C absolute acetone, reacted with affinity purified anti-CaM, washed with PBS and reacted with rhodamine-conjugated goat anti-rabbit IgG. After washing, the cells were observed using a Zeiss photomicroscope equipped for UV epi-illumination. The cell-surface distribution of FITC-Con A and the intracellular localization of rhodamine-labelled anti-CaM could be separately visualized on or in the same cell by switching to the appropriate excitation filter, beam splitter and barrier filter (Zeiss BP 450-490, FT 510 and BP 520-560 for FITC and BP 456/10, FT 580, LP 590 for rhodamine). a-c, WiL2 cell challenged with FITC-Con A, immediately fixed and treated with preimmune antiserum followed by rhodamine-labelled goat anti-rabbit IgG showing the diffuse ring-like surface localization of FITC-Con A receptors (b) and the control level of background rhodamine fluorescence (c). The same level of back ground fluorescence was observed when the primary antibody was omitted entirely or when anti-CaM was first preabsorbed with excess CaM. d-f, WiL2 cell treated as in a-c but first allowed to cap the FITC-Con A receptors (e) at 37 °C before fixation, again illustrating the control level of background rhodamine fluorescence (f). g-i, WiL2 cell challenged with FITC-Con A, immediately fixed and treated with the affinity purified anti-CaM IgG followed by rhodamine-labelled goat anti-rabbit IgG. The FITC-Con A is diffusely located at the cell surface (h) while the rhodaminelabelled anti-CaM is diffuse throughout the cytoplasm (i). j, k, WiL2 cell treated as in g-i but first allowed to begin capping the FITC-Con A receptors at 37 °C for 30 min before fixation. Note the lateral redistribution of FITC-Con A receptors at the cell surface (k) accompanied by a dramatic reorganization of the intracellular anti-CaM binding sites beneath the capping FITC-Con A (1). m-o, WiL2 cell treated as in g-i but first allowed completely to cap the FITC-Con A for 1 h before fixation. Here the rhodamine-labelled anti-CaM has reorganized within the cytoplasm (o) beneath the tight FITC-Con A cap (n). The same pattern of specific anti CaM localization was observed when whole immune serum was substituted for affinity purified serum. Each row, left to right, shows the same cell. a, d, g, j, m, Light micrograph; b, e, h, k, n, FITC-Con A fluorescence; c, f, i, l, o, rhodamine fluorescence. All  $\sim \times 1,460$ . Fluorescence micrographs were recorded using Tri-X (Kodak) film and developed in Diafine for 5 min.

control cells labelled with preimmune serum (Fig. 2c). We interpret this to indicate that CaM is uniformly distributed throughout the unstimulated cell in which Con A receptors are diffuse and that receptor-mediated endocytosis is occurring only at a basal rate. When the cells are allowed to cap the FITC-Con A at 37 °C for 30 min before fixation (Fig. 2j-l) a dramatic redistribution of intracellular anti-CaM binding sites occurs. As FITC-Con A receptors begin to move laterally towards one region of the cell surface (Fig. 2k), the originally diffuse anti-CaM binding sites also move to regions in the cytoplasm just beneath the large clustered regions of Con A and sometimes also appear at a focal region near the cell centre (Fig. 21). Control cells allowed to cap FITC-Con A (Fig. 2e) and then treated either with preimmune serum instead of anti-CaM, omitting primary antibody, or by preabsorbing anti-CaM with excess CaM, still show only diffuse low-level rhodamine staining

When cells are allowed to cap the FITC-Con A completely (Fig. 2n), rhodamine-labelled anti-CaM binding sites become further reorganized in the cytoplasm beneath the cap (Fig. 2o). This suggests that after ligand challenge, CaM is bound to and moves with some cytoplasmic structure that itself co-caps with the ligand. As indirect immunofluorescence studies<sup>19</sup> indicate that actin and myosin co-cap with certain surface receptors and co-isolate with Con A caps from *Dictyostelium*<sup>20</sup>, one potential CaM binding structure is the microfilament network of the cell cortex. Alternatively, the finding that endocytosis accompanies capping and is sensitive to CaM-directed drugs<sup>3</sup> and that CaM co-isolates with coated vesicles from WiL2 cells<sup>3</sup>, suggests that a



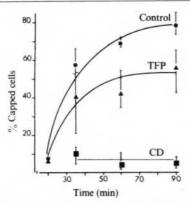


Fig. 3 Kinetics of capping after challenge with Con A and the effects of cytochalasin D (CD) and trifluoperazine (TFP) on capping. WiL2 cells were incubated in PBS ( $\bullet$ ), cytochalasin D (Sigma),  $10 \,\mu g \, \text{ml}^{-1}$  ( $\blacksquare$ ), or trifluoperazine (Smith Kline & French),  $15 \,\mu \text{M}$  ( $\blacktriangle$ ), for 10 min, challenged with  $10 \,\mu g \, \text{ml}^{-1}$  FITC-Con A and allowed to cap the receptor-ligand complexes at  $37 \,^{\circ}\text{C}$ . At various times after ligand challenge, cells were fixed and fields of 20--30 cells photographed using the fluorescence microscope. Each point represents the mean value of % capped cells for a minimum of  $100 \, \text{cells}$ , the bars represent the maximum range for individual\_fields. A capped cell is defined here as one which has redistributed the FITC-Con A, clearing at least half of its cell surface of label.

second potential CaM binding site is the membrane of clathrincoated pits and vesicles. We have investigated these suggestions in a series of experiments using drug inhibitors of capping and endocytosis.

We have previously reported<sup>3</sup> that the cytochalasin derivative dihydrocytochalasin B inhibits capping of cell-surface IgM by WiL2 cells. However, it has no apparent effect on the recruitment of clathrin coat material to ligand–IgM complexes and only partially reduces endocytosis of the complexes<sup>3</sup>. Similarly, although CD (10 µg ml<sup>-1</sup>) inhibits Con A capping almost completely (Fig. 3), this drug does not inhibit eventual endocytosis of ligand (to be published elsewhere).

Trifluoperazine dihydrochloride at low concentration (10–25 μM) is a potent and highly specific inhibitor of CaM-mediated processes<sup>21–24</sup>, and binds to CaM with high affinity in the presence of Ca<sup>2+</sup> (refs 22, 23). It inhibits CaM stimulation of brain phosphodiesterase<sup>22,23</sup> and adenylate cyclase<sup>23</sup>, inactivates CaM regulation of smooth muscle contraction<sup>24</sup>, and in WiL2 cells, significantly blocks entocytosis of surface receptors<sup>3</sup>. An important effect of TFP is the inhibition of clathrin recruitment to clustered receptor complexes, which suggests that clathrin recruitment is a CaM-regulated event<sup>3</sup>. TFP does not prevent capping of either Con A receptors (Fig. 3) or receptor-IgM in WiL2 cells<sup>3</sup>, in contrast to its effect in other cells<sup>25</sup>, but it does reduce the final percentage of tightly capped cells by about one-third (Fig. 3).

The differential effects of CD and TFP on receptor capping have allowed us to explore whether CaM redistribution on ligand challenge is obligately coupled to capping. Figure 4a-c illustrates the reorganization of intracellular CaM during capping by WiL2 cells in normal conditions and corresponds to Fig. 2j-l. When WiL2 cells are incubated with CD (10 µg ml<sup>-1</sup>) but otherwise treated as in Fig. 4a-c, Con A receptor capping is inhibited (Figs 3, 4e). The FITC-Con A is patched and by 30 min is mostly still at the cell surface although it is eventually internalized3. Anti-CaM binding sites are not diffuse in the CD-treated cells. Rather, rhodamine-labelled anti-CaM appears as numerous 'hot spots' in the cytoplasm (Fig. 4f), many of which lie directly beneath patches of FITC-Con A at the cell surface (Fig. 4e, f). In the absence of ligand challenge, CD treatment does not result in CaM redistribution (not shown). Therefore, CaM reorganization follows ligand challenge and does not depend on receptor capping. Receptor capping and CaM redistribution are separable events.

When WiL2 cells are incubated with TFP (15  $\mu$ M) and treated as in Fig. 4a-c, Con A receptor capping still occurs (Fig. 4h) in most of the cells, albeit in a lower percentage than the controls (see Fig. 3). Nevertheless, in these TFP-treated capped cells

anti-CaM staining is diffuse throughout the cell (Fig. 4i). The rhodamine-labelled anti-CaM is considerably brighter in TFP-treated cells than in any other treatment. We do not know why this is so; in these conditions TFP autofluorescence is undetectable. Perhaps TFP binding to CaM in the cytoplasm unmasks antigenic determinants on the molecule. In any event, these results clearly show that capping can occur in the presence of TFP, independently of CaM redistribution.

To probe the effect of Ca<sup>2+</sup> on these processes, we have begun chelator experiments. When WiL2 cells are incubated in conditions where the external free Ca<sup>2+</sup> concentration is held below 10<sup>-7</sup> M by inclusion of the calcium chelator EGTA in the external medium, capping of Con A receptors proceeds normally (Fig. 4k), in agreement with the findings of other workers<sup>2</sup>. In these conditions anti-CaM binding sites again remain diffuse (Fig. 4l). These results suggest that Ca<sup>2+</sup> is a necessary intermediate in the coupling of capping and CaM redistribution.

The observations reported here, taken with our earlier studies<sup>3</sup>, support the suggestion that receptor-mediated endocytosis operates under the control of CaM-Ca<sup>2+</sup> signals that involve transient fluxes of Ca<sup>2+</sup> across the plasma membrane at sites of ligand-receptor clusters. In our experiments the structural redistribution of CaM after ligand challenge and presumably CaM-Ca<sup>2+</sup> complex formation corresponds to the formation and distribution of coated endocytotic vesicles rather than to microfilaments. In this regard, it is important to recall that CaM appears to be an intrinsic component of coated vesicles isolated from WiL2 cells<sup>3</sup> and from brain<sup>26</sup>. Although in normal conditions coated vesicle formation is closely coupled to microfilament formation and function, this coupling is not

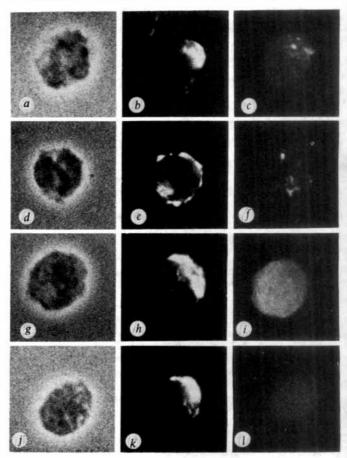


Fig. 4 Receptor capping and CaM redistribution are separable events. WiL2 cells were incubated in PBS (a-c), cytochalasin D,  $10 \mu g \text{ ml}^{-1} (d-f)$ , trifluoperazife,  $15 \mu M (g-i)$  or EGTA, 5 mM (j-i) for  $10 \min$ , challenged with  $10 \mu g \text{ ml}^{-1}$  FITC-Con A and allowed to cap the receptor-ligand complexes for 30 min at  $37 \, ^{\circ}\text{C}$ . Cells were fixed and then processed for anti-CaM staining as described in Fig. 2 legend. Each row, left to right, shows the same cell. a, d, g, j, Phase micrographs; b, e, h, k, FITC-Con A fluorescence; c, f, i, l, anti-CaM rhodamine fluorescence. All  $\sim \times 1,460$ .

obligatory. Two sites of action of CaM-Ca<sup>2+</sup> are interesting potential control points in this process: (1) The activation of myosin light chain kinase for acceleration if capping. This might explain the partial inhibition of capping seen on TFP treatment. (2) The recruitment of clathrin coats to the membrane

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## Long-term human T-helper lines producing specific helper factor reactive to influenza virus

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Our understanding of the mechanism of T-cell help in antibody production in the mouse has been improved by the production of long-term lines of T cells, either using T-cell growth factor (TCGF or IL-2) or by creating T-cell hybridomas. Such cells have been shown to produce non-antigen-specific 1,2 or antigenspecific helper factors3 with genetically restricted or unrestricted activity<sup>1-3</sup>. However, the factors are still not well characterized. We have examined the role of human T-helper cells and their soluble factors in inducing antibody production, initially in a xenogeneic system, using mouse B cells4. Using a human in vitro secondary antibody response system for influenza virus we have investigated the effect of helper factors on human B cells. We report here the production of long-term cultures of antigenspecific human helper cells. Both the cells and the high-potency antigen-specific helper factor (HF) which they produce act on human B cells in a genetically restricted manner. Preliminary mapping of the restriction indicates associations, but not identity, with HLA-DR.

Table 1 Cell membrane phenotype of F, T-cell line

	% Of posi	tive cells
Marker	Day 40	Day 150
UCHT1	93	98
OKT1	ND	98
Leu 2a	0	3
Leu 3a	83	82
HLA-DR	72	40
OKM1	ND	2
BA-1	ND	2
SIg	0	0
E rosettes	ND	>99

Human peripheral blood lymphocytes (PBLs) were prepared on a Ficoll-Hypaque gradient, then suspended at  $10^{-7}\,\mathrm{ml^{-1}}$  in RPMI 1640 containing 10% fetal calf serum (FCS) and mixed with two volumes of 8-(aminoethyl isothiouronium bromide hydrobromide)-treated sheep red blood cells (SRBC). After incubation at 4 °C, E\* cells (T cell-enriched suspensions) were separated from non-rosetting cells (E\*; T cell-depleted suspensions) by centrifugation through a Ficoll–Hypaque gradient. Red cells were removed by hypotonic shock. E $^*$  cells were cultured for 7 days at a concentration of  $1\times10^6$  ml $^{-1}$  with A/X31 (5  $\mu g$  ml $^{-1}$ ) in the presence of 10% horse serum in 50-ml Falcon flasks. Dead cells were then removed on a Ficoll-Hypaque gradient and the cells (containing 20-40% blasts) were re-cultured at a concentration of  $1\times10^5$  ml $^{-1}$  in RPMI+10% FCS supplemented with TCGF medium diluted to an optimal concentration (~25%). The supernatants were prepared by culturing  $1\times10^6$  pooled human tonsil cells from different donors with  $2~\mu g~ml^{-1}$  PHA in RPMI-5% human serum for 48 h. PHA was removed from TCGF medium by passage over a column of Sepharose 4B to which rabbit anti-PHA immunoglobulin had been coupled<sup>17</sup>. PHA-depleted medium was no longer able to induce the proliferation of PBLs. TCGF medium was filtered and stored at -20 °C. Every 3-4 days, medium was removed by centrifugation and cells re-seeded at  $1 \times 10^5$  cells ml<sup>-1</sup> in fresh TCGF medium in the absence of antigen.  $F_7$  T cells have been kept growing for >4 months. The average doubling time is 48 h. Aliquots of  $F_7$  cells  $(2\times10^5)$  were incubated for 30 min on ice with saturating concentrations, previously determined by titration, of monoclonal antibodies (culture supernatant or purified immunoglobulin) washed three times in HEPES-buffered RPMI medium with 5% FCS and stained for 30 min on ice with human immunoglobulin-absorbed and immunoabsorbentpurified fluorescein isothiocyanate-conjugated (FITC) sheep anti-mouse immunoglobulin antiserum. After a further three washes the cells were either examined by fluorescence microscopy or analysed by Becton-Dickinson FACS I or IV. Controls and precautions to minimize non-specific binding have been described previously<sup>18</sup>. Monoclonal antibodies used were: UCHT1 (against all mature peripheral human T lymphocytes<sup>18</sup>); Leu 2a and 3a (gifts from Becton-Dickinson); anti-HLA-DR (DA2)<sup>19</sup> (gift from Dr M. Crumpton); OKM1 and OKT11 (gifts from Dr G. Goldstein); and BA-1 (ref. 6) gift from Dr J. Kersey. Polyspecific FITC sheep anti-human immunoglobulin antiserum was prepared in our laboratory—this brightly stains human peripheral blood B lymphocytes. ND, not determined.

Peripheral blood lymphocytes from donors (laboratory volunteers) known to respond to strain A influenza virus (A/X31) and strain B, Hong Kong B virus (HKB), were cultured with A/X31 for 7 days before addition of human TCGF depleted of phytohaemagglutinin (PHA). Several lines have been obtained which, after 4-6 weeks of growth, displayed stable characteristics. The surface characteristics of one of these cell lines, F<sub>7</sub>, are shown in Table 1. After 6 weeks, essentially all the cells were T cells (UCHT1+, E+, and OKT11+, OKM1-) of the helper type Leu 3a<sup>+</sup>, 2a<sup>-</sup> (see Table 1). A high, but variable proportion of cells stained with antisera to HLA-DR throughout culture. No staining was seen with conventional anti-immunoglobulin antisera or the monoclonal anti-B lymphocyte serum BA-1 (ref. 6). Analysis of these lines earlier in their development revealed different results, in that some cells were Leu 3a<sup>-</sup>, 2a<sup>+</sup>, but these cells were lost spontaneously during culture.

The helper activity of the T-cell lines was assayed on autologous B cells and monocytes using erythrocyte rosette-depleted cells' in the absence of TCGF. In a typical experiment (Fig. 1) with the F<sub>7</sub> cell line, 65 days after initiation of culture, optimal help was obtained with  $5 \times 10^2$  cells, compared with  $10^5$  fresh T cells from the same donor (before culture). Moreover, the number of antigen-responding helper T cells was  $\sim 1:10-1:30$ , as measured by limiting dilution, which is in the range of that observed for a cloned helper T-cell line in the mouse2. Note that in this and most other experiments, excess helper line cells markedly inhibited the response. The helper property of the cell

line remained after 5 months of culture. The specificity of the helper line was demonstrated by the markedly diminished capacity to respond to HKB compared with uncultured T cells (Fig. 1). Specificity was verified by the lack of a response to varicella-zoster virus, and in 'bystander' experiments with mixtures of A/X31 and HKB (data not shown).

We investigated the mechanisms of help by the cell lines. The best studied— $H_2$  (a similar cell line from another donor) and  $F_7$ —yield high potency supernatants. These could be obtained from T cells cultured alone without TCGF, but higher titres of HF were obtained when autologous irradiated  $E^-$  cells plus antigen (A/X31) were added (Fig. 2). Again, maximum help was obtained at low percentages of HF (0.1–1%), whereas higher concentrations were inhibitory. This help was specific, as the response of HKB was not influenced. Supernatants produced in the presence of A/X31 also demonstrated no bystander effect, as previously noted for mouse TCGF lines<sup>7</sup>. (This effect is the induction by specific antigen of a nonspecific factor which

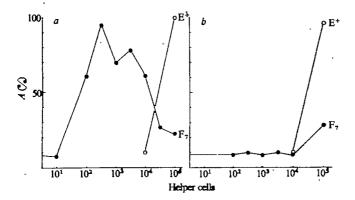


Fig. 1 Helper activity effected by A/X31-specific F<sub>7</sub> T-cell line. The method described by Callard and modified by Zanders et al. was used to assay the ability of washed TCGF-free F7 cells to help E cells in producing antibodies to A/X31. E and E cells were separated by centrifugation through a Percoll (Pharmacia) d = 1.08gradient after incubation at 4°C with AET-treated SRBC. Red cells were removed by hypotonic shock. E cells (1×105) were cultured in round-bottomed 96-well microtitre plates with various amounts of either fresh  $E^+$  or  $F_7$  T (after 65 days of culture) cells in 200  $\mu$ l of RPMI-1640 (Gibco) supplemented with 10% horse serum and glutamine (200 mM), and A/X31 (1  $\mu$ g ml<sup>-1</sup>; a), or HKB (0.2 µg ml<sup>-1</sup>; b). Cultures performed in triplicate were maintained at 37 °C for 6 days, then washed and recultured in 0.1 ml RPMI medium containing 5% FCS and glutamine. Supernatants were collected after 24 h and assayed for anti-A/X31 or anti-HKB antibodies by a solid-phase enzyme immunoassay. Antibody production was determined by reading the absorbance at 405 nm of the transformed substrate of alkaline phosphatase; actual amounts of antibody were calculated by referring to a standard curve<sup>20</sup>. Results are expressed as a percentage of the optimal response induced by 10<sup>3</sup> E<sup>+</sup> cells. For each experimental point, the s.d. was <25% of the mean value. The amount of antibody detected in these experiments ranged from 45.4 to 114 ng ml<sup>-1</sup> (100% A). Backgrounds (E<sup>-</sup> alone) ranged from 4 to 11% of maximal values.

induces B cells to respond, if their appropriate antigen is present.) However, high concentrations of  $F_7$  cells or HF from the supernatant of  $F_7$  ( $S_7$ ) sometimes gave small but significant nonspecific help, as shown in Figs 1, 2.

Most reports of antigen-specific HFs indicate that they are not genetically restricted in their action (for review see ref. 8) but some HFs do seem restricted<sup>9,10</sup>. This was investigated using E-cells (a mixture of B cells and monocytes) which were either autologous, HLA-DR-semi-compatible or incompatible with the  $F_7$  cells. HF derived from two cell lines ( $H_2$  and  $F_7$ ) showed a genetically restricted helper activity stimulating only B cells of certain donors. As shown in Table 2 for  $F_7$  cells and helper factor ( $S_7$ ), autologous and both parental B cells were helped, whereas

Table 2 Restriction of the help effected by F7 cells and supernatants

E	Sou	arce of help (%)		
(DR antigen shared	None	E <sup>+</sup>		
with F <sub>7</sub> )	(E cells alone)	(autologous)	$F_7$	S <sub>7</sub>
4, 6 (autologous)	6	100	93	62
4, 6 (unrelated)	5	100	82	104
4 (perent (3, 4)	7	100	41	47
4 (1/4)	' 8	100	96	91
4 (3/4)	5	100	79	92
4 (3/4)	14	100	99	90
4 (4/10)	7	100	85	68
4 (4/5)	10	100	74	73
4 (4/7)	8	100	93	90
4 (4/7)	7	100	77	68
6 (perent) (6/7)	6	100	48	42
6 (6/7)	5	100	7	7
6 (1/6)	5	100	10	12
6 (2/6)	7	100	12	12
6 (3/6)	12	100	74	94
None (3/7)	9	100	11	7
None (2/7)	5	100	7	10
None (3/7)	11	100	10	14
None (2/-)	8	100	15	9
None (2/7)	4	100	88	62
None (2/-)	10	100	12	7
None (2/7)	9	100	8	11
None (2/10)	9	100	8	11
None (1/-)	6	100	8	5

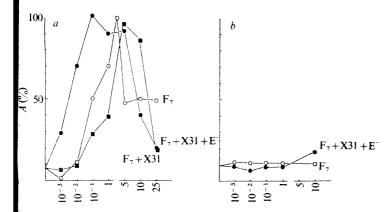
Either  $F_7$  T cells or supernatants (prepared in the presence of irradiated  $E^-$  cells and A/X31) were assayed as described previously  $^{20}$  on  $E^-$  cells sharing either two, one or no HLA-DR antigens with  $F_7$  T cells. The data are expressed as a percentage of the optimal response provided by  $E^+$  cells autologous to the relevant  $E^-$  cells. For each donor the maximal help provided by the optimal number of  $F_7$  T cells and optimal concentration of supernatant  $(S_7)$  is given. Each individual was HLA typed on two occasions with identical results. All donors were tested at least twice over a wide range of cell and factor concentrations. The 2/7 responder has been tested 4 times with  $F_7$  cells and twice with  $S_7$ .

most HLA-DR-incompatible donor B cells (8 out of 9) were not. All HLA-DR4 B cells tested (10) were strongly helped by  $F_7$  cells and  $S_7$  HF, suggesting either identity or a strong linkage disequilibrium between the restriction element and DR4, as only one of the DRW6-positive B cells except for the parental DR6/7 cells were helped. The latter result could be explained by the wide heterogeneity of the DRW6 group 11. However, B cells from one of nine DR-incompatible donors were consistently helped by  $F_7$  to produce anti-A/X31 antibodies.

This indicates a close association, but not indentity, between the locus coding for T-B restriction and the HLA-DR locus. The nature of the component expressing the genetic restriction is unknown, but as HLA-DR antigens seem to have homology with I-E/C products<sup>12</sup>, the restricting element detected in our system may be the equivalent of the mouse I-A region, which is known to be intimately involved in T-helper cell and factor function<sup>3,13</sup>. The p29, 34 molecule recently described by Nadler et al.<sup>14</sup> would be a likely candidate.

The results obtained with long-term cultured T-cell lines specific for influenza strain A/X31 indicate clearly that, just as in the mouse, antigen-specific helper cells can be propagated long term (for over 4 months) using TCGF. A report of human, non-antigen-specific T cells has been published elsewhere15 The type of helper cell cultured varies: Schreier and Tees 1.7 and Watson<sup>2</sup> have reported erythrocyte-specific T cells which, while responding to antigen specifically, did not help in a specific manner. In contrast, Eshar et al.3 described a helper line specific for poly(L-tyrosine-L-glutamic acid)-poly(D,L-alanine)-poly(Llysine) (TGAL) and P. Erb (personal communication) has derived a keyhole-limpet haemocyanin-specific helper line which produces antigen-specific HF. It seems likely that these contrasting results, which closely parallel discrepancies concerning mechanisms of T-cell help, reflect different subpopulations of helper cells.

Our results have been obtained with a long-term cell line, and not with clones. Many attempts to clone these cells at low numbers, in the presence of feeder cells, have failed. It is



Concentration of F7 supernatant

Fig. 2 Helper activity of F<sub>7</sub> supernatants. Long term-cultured T cells (F<sub>7</sub>) after 80 days of culture  $(1 \times 10^6 \text{ ml}^{-1})$  were incubated cells  $(F_7)$  after 80 days of culture  $(F_7)$  and  $(F_7)$  alone (O) or with optimal dose of antigen A/X31  $(\blacksquare)$  or with a strength of the 18 h in TCGF-free medium containing 10% horse serum. The supernatants were collected by centrifugation, filtered and stored at -20° C. They were assayed at various concentrations (expressed as a percentage) for their ability to help the production in vitro of anti-A/X31 (a) or anti-HKB (b) by autologous E cells, as described in Fig. 1 legend. Results are expressed as a percentage of the optimal response induced by 10° E<sup>+</sup> cells.

conceivable that these helper cells will not grow in the absence of another 'amplifier cell', also of the 3a+ phenotype.

The genetically restricted nature of the specific helper factor obtained here is intriguing, and is comparable with other results in the mouse<sup>10</sup> and with the specific nature of the help for anti-tetanus antibody production in humans<sup>16</sup>, but is in contrast to many others<sup>8,13</sup>. In view of the genetic restriction repeatedly observed in T-B collaboration, we feel that further analysis of these cell lines should elucidate the nature of T-cell receptors and their factors, and the mechanisms of T-B collaboration.

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# Antibody directed at a surface structure inhibits cytolytic but not suppressor function of human T lymphocytes

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Experiments using conventional antisera<sup>1,2</sup> and monoclonal antibodies3-5 have shown that there are two functionally distinct human T-cell subsets which express unique cell-surface glycoproteins<sup>6-9</sup>. The inducer population expresses a 62,000molecular weight  $(M_r)$  antigen termed T4 (ref. 6) whereas the cytotoxic/suppressor population lacks T4 but expresses T5 antigen. This antigen is a 76,000-M, molecule in nonreducing conditions and a 33,000-M<sub>r</sub> glycoprotein occasionally appearing as a doublet in reducing conditions. As the functions of subset-specific T-cell surface glycoproteins have not been elucidated, we have now examined the effect on T-cell function three monoclonal antibodies reactive with the cytotoxic/suppressor subset; anti-T8, antibody markedly inhibited cell-mediated lympholysis (CML) and anti-T8 partially affected CML, whereas anti-T5 had no effect. In contrast, there was no effect on suppressor cell function. Immunoprecipitation studies and competitive binding experiments indicated that anti-T8 and anti-T8A, like anti-T5, defined a 33,000-M, antigen. Taken together, our results suggest that of a possible of  $33,000-M_r$  glycoproteins on the cytotoxic/suppressor T-lymphocyte subset, that which is reactive with anti-T8<sub>A</sub> has some effect on the cytolytic mechanism.

Effector function for CML resides in the subset of T lymphocytes recognized by the antibodies anti-T5 and anti-T8 (refs 3, 4, 9), which are monoclonal hybridoma products of IgG1 and IgG2a isotypes, respectively<sup>4,5</sup>. Elimination of the subsets of cells reactive with these antibodies by fluorescence-activated cell sorting (FACS) or, in the case of anti-T8, complementmediated lysis, was found to abrogate the cytotoxic effector function of the unfractionated T-cell population9. Here, an additional monoclonal antibody, anti-T8A, was developed by standard hybridoma techniques after immunization of a BALB/cJ mouse with human thymocytes as described previously<sup>10</sup>. Hybridoma cultures containing antibody reactive with E<sup>+</sup> or thymocyte populations or both were selected, cloned and recloned by limiting dilution methods in the presence of feeder cells. Malignant ascites were then developed and used for analysis. Anti-T8<sub>A</sub> represents an ascites from one hybridoma clone (SFCI 21Thy). The pattern of reactivity of the anti-T8<sub>A</sub> was identical to that described for anti-T8 (ref. 5) as it defined -80% of thymocyte and 30-35% of peripheral T cells and was unreactive with B cells, null cells and macrophages by indirect immunofluorescence.

To determine whether the antigens defined by these antibodies were themselves important for the cytotoxic response of T lymphocytes, the following procedure was used. T cells were sensitized to alloantigens in mixed lymphocyte culture (MLC) for 6 days. Subsequently, the cytotoxic cells (CTL) were collected, incubated with a 1:1,000 dilution of a given monoclonal antibody or control and their ability to lyse 51 Cr-labelled target cells was investigated. In the absence of monoclonal antibody (control), specific lysis in primary CML was ~28-29% at either

Table 1 Effect on % specific lysis of preincubation of CTL with monoclonal antibodies

		Specific lysis at ctor/target ratio	
Monoclonal antibody	40 · 1	20:1	10:1
Control Anti-T5 Anti-T8 Anti-T8 <sub>A</sub>	28±2 30±2 16±2 2±1	29±1 28±2 14±2 2±1	$21\pm1$ $22\pm2$ $15\pm1$ $0.7\pm1$

Peripheral T cells were activated in one-way MLC with mitomycin C-treated allogeneic, human mononuclear cells as previously described 10. Subsequently, alloantigen-activated killer cells were collected and tested in a 51 Cr microcytotoxicity assay for 4 h (ref. 3). Specificity of CML was tested by using an additional allogeneic target population unrelated to the mononuclear population used in the initial sensitization phase of MLC. The level of nonspecific killing was always <10% of specific killing. To determine whether anti-T5, anti-T8 or anti-T8<sub>A</sub> antibody inhibited CML in the absence of complement, some killer cells were incubated with a 1:1,000 dilution of monoclonal antibody or control ascites at 20 °C for 30 min before addition of the relevant target cells. The control ascites was obtained from BALB/cJ mice immunized with a non-producing hybridoma clone 10.

a 40:1 or 20:1 effector/target cell ratio but was reduced to 21% at a 10:1 effector/target ratio (see Table 1). Preincubation of cytotoxic cells with anti-T5 did not affect the magnitude of this response, whereas preincubation of 6-day MLC-stimulated cells with anti-T8 resulted in a diminished percentage specific lysis such that, even at a 40:1 effector/target cell ratio, only 16% of target cells were killed. A similar percentage lysis was obtained if the effectors were preincubated with anti-T8 and used at an effector/target ratio of 20:1 or 10:1. In contrast, the anti-T8A monoclonal antibody almost completely inhibited killing. These findings indicate that anti-T5 has no inhibitory effect on the effector phase of CML whereas the anti-T8 and anti-T8A monoclonal antibodies have a moderate to marked effect, respectively. These effects were seen at dilutions of monoclonal antibody between  $10^{-2}$  and  $10^{-4}$ . As anti-T5 and anti-T8<sub>A</sub> monoclonal antibodies are of the IgG1 subclass<sup>3</sup>, the results cannot be explained by differences in immunoglobulin isotype.

Anti-T8<sub>A</sub> had no effect on CTL effector function when added at the initiation of MLC (data not shown). In contrast, anti-T3 regularly blocked CTL generation in MLC and generally also blocked at the effector phase<sup>11-15</sup>. The observation that anti-T3, which defines a 19,000-M<sub>r</sub> glycoprotein, and anti-T8<sub>A</sub>, which defines a 33,000-M<sub>r</sub> protein, both inhibit cytolysis suggests that multiple cell surface antigens are important in mediation of CML.

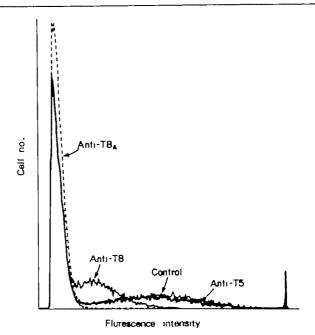


Fig. 1 Competitive blocking studies with monoclonal antibodies to cytotoxic/suppressor cells. The relationships between anti-T5, anti-T8 and anti-T8<sub>A</sub> antibodies were examined in competitive binding studies by direct immunofluorescence using FACS<sup>10</sup>. T cells (1×10<sup>5</sup>) were treated with 0.15 ml of a 1:100 dilution of either control ascites, anti-T5, anti-T8 or anti-T8<sub>A</sub>, incubated at 4 °C for 30 min and washed twice. The control ascites was obtained from BALB/cJ mice (see Table 1). The cells were reacted with 0.15 ml of a 1:50 dilution of directly fluoresceinated anti-T8<sub>A</sub> at 4 °C for 30 min, centrifuged and washed three times. Subsequently, 40,000 cells were analysed on the FACS I (Becton Dickimson) and the intensity of fluorescence per cell recorded on a pulse height analyser.

As T5(T8<sup>+</sup>) T cells have been observed to suppress pokeweed mitogen (PWM) induced immunoglobulin synthesis, we examined whether antibodies to the T5, T8 or T8<sub>A</sub> antigen could affect this suppressor function. B cells and T4<sup>+</sup> inducer T cells were cultured with various numbers of T8<sup>+</sup> T cells in the presence of the particular monoclonal antibody and stimulated with PWM. Immunoglobulin production was measured by radioimmunoassay after 7 days of culture. As expected (Table 2), T4<sup>+</sup> T cells induced B-cell immunoglobulin production whereas T8<sup>+</sup> T cells did not (11,000 as opposed to <200

Table 2	Anti-T5	-T8 and -T87	antibodies do n	ot inhibit suppressor	T-cell function	in T-B interactions
	THU-ID	-10 444 -101		OF TITITION SUPPLY CHACK	I -CCII I UII CUOII	

	Cells in PWM cultu	<del></del>	IgG	(ng ml <sup>-1</sup> ) secreted or or absence of mor	ver 7 days in the pre- nocional antibodies	sence
B celis	T4 <sup>+</sup> cells added	T8 <sup>+</sup> cells added	Control	Anti-T5	Anti-T8	Anti-T8 <sub>A</sub>
+	0	2×10 <sup>4</sup>	<200	<b>≤</b> 200	<200	<200
, +	2×10 <sup>4</sup>	0	11,800	11,400	11,600	11,400
. +	2×10 <sup>4</sup>	1×10 <sup>4</sup>	8,500	8,600	8,400	8,300
· +	2×10 <sup>4</sup>	5×10 <sup>4</sup>	4,000	4,100	4,300	3,900
+	2×10 <sup>4</sup>	10×10 <sup>4</sup>	300	400	<200	<200
-	2×10 <sup>4</sup>	0	<200	<200	<200	<200

Unfractionated mononuclear cells were separated into T- and B-enriched populations as previously described. The B-cell population was <1% E-rosette positive. The T4<sup>+</sup> and T8<sup>+</sup> T-cell subsets were obtained by complement-dependent lysis of unfractionated T cells with anti-T8 and anti-T4, respectively, and were ≥93% pure on re-analysis. To 5×10<sup>4</sup> B cells or final medium (RPMI 1640 supplemented with 20% heat-inactivated fetal calf serum (Microbiological Associates), 200 mM L-glutamine, 25 mM HEPES buffer (Microbiological Associates), 0.5% sodium bicarbonate and 1% penicillin-streptomycin) were added varying numbers of T4<sup>+</sup> and T8<sup>+</sup> T cells in a total volume of 0.1 ml with 0.1 ml PWM (Gibco) diluted 1:50 in final medium containing control ascites (1:100), anti-T5 (1:100), anti-T8 (1:100) or anti-T8<sub>A</sub> (1:100). After 7 days in culture at 37 °C in a humid atmosphere with 5% CO<sub>2</sub>, culture supernatants were collected and IgG secretion during the 7-day period determined by solid phase radioimmunous y.

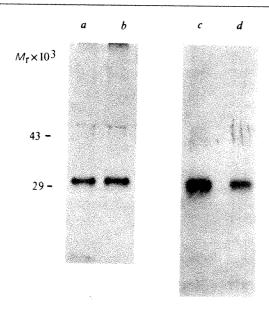


Fig. 2 Characterization and isolation of the T5, T8 and T8<sub>A</sub> antigens. The external proteins of the human T-cell line HPB-ALL were labelled with 125I using IODO-GEN (Pierce). Labelled cells washed three times with phosphate-buffered saline containing inhibitor trypsin (Sigma), 2 mM phenylmethylketonesulphone (PMKS) and 1 mM iodioacetamide (Pierce) were extracted with 1% Nonidet-P40 (NP40; Partide Data Laboratories, Elmhurst, Illinois) in 0.01 M Tris-HCl pH 8.0, 2 mM PMKS, trypsin inhibitor and 1 mM iodoacetamide at 0 °C for 30 min. Nuclear debris was removed in an Eppendorf centrifuge (15 min at 12,000g) and the supernatant cleared in an Airfuge (30 min at 100,000g; Beckman). Immunoprecipitations were carried out by a technique which uses preformed complexes prepared from rabbit anti-mouse IgG serum and the monoclonal reagent anti-T5, anti-T8 or anti-T8<sub>A</sub> as described elsewhere<sup>6</sup>. Immunoprecipitates were washed with 0.01 M Tris-HCl pH 8.0, 0.59% deoxycholate, 2 mM PMKS and 0.01 M Tris-HCl pH 8.0, 0.2% NP40. The surface antigens immunoprecipitated with the anti-T5, anti-T8 and anti-T8<sub>A</sub> monoclonal antibodies were analysed by SDS-polyacrylamide gel electrophoresis on 5-15% gradient gels. a, Anti-T8; b, anti-T8<sub>A</sub>; c, anti-T8; d, anti-T5.

ng ml<sup>-1</sup>, respectively, secreted over 7 days). Furthermore, when increasing numbers of T8+ T cells were added to a mixture of  $2 \times 10^4 \,\mathrm{T}^{4+} \,\mathrm{T}$  cells and  $5 \times 10^4 \,\mathrm{B}$  cells, immunoglobulin production was markedly suppressed. When  $5 \times 10^4 \text{ T8}^+\text{ T}$  cells were added to the T4+B cell mixture, immunoglobulin production decreased by >50% to 4,000 ng. IgG secretion was virtually abolished (300 ng ml<sup>-1</sup>) when  $1\times10^5$  T8<sup>+</sup> T cells were added to the T4<sup>+</sup>-B cell population. More importantly, preincubation of the mixture of T and B cells with anti-T5, anti-T8 or anti-T8A had no effect on the suppression caused by the T8<sup>+</sup> T-cell subset. Thus, it seems that the inhibitory effect of anti-T8<sub>A</sub> is selective for cytotoxic effector, rather than suppressor, function. However, the two assays are not comparable as the former occurs over 4 h and the latter over 7 days.

To determine whether these monoclonal antibodies defined related or distinct sets of antigens, the ability of one monoclonal antibody to inhibit binding of a second was determined by direct immunofluorescence. As shown in Fig. 1, ~30% of peripheral T cells were reactive with fluorescein isothiocyanate-conjugated (FITC)-labelled anti-T8<sub>A</sub> in a FACS. Preincubation of T cells with unlabelled anti-T8<sub>A</sub> before addition of FITC-anti-T8<sub>A</sub> totally inhibited the binding of the latter. In contrast, preincubation with unlabelled anti-T5 was not inhibitory. Preincubation of T cells with anti-T8 antibody showed a partial inhibition of FITC-anti-T8<sub>A</sub> binding. Similarly, preincubation of T cells with anti-T8<sub>A</sub> inhibited binding of FITC-anti-T8. These results suggest that the determinant defined by anti-T5 is

distinct from that defined by anti-T8<sub>A</sub> and that anti-T8 and anti-T8A seem to react with a related structure.

The molecular nature of these antigens was then examined in a second series of experiments. Solubilized membrane preparations were obtained from the externally labelled human T-cell line HPB-ALL and the antigens defined by anti-T5, anti-T8 and anti-T8<sub>A</sub> antibodies precipitated and electrophoresed on SDS-polyacrylamide gels. As previously reported<sup>6</sup>, the antigen precipitated by anti-T5 antibody was a 33,000-M<sub>r</sub> protein in reducing conditions (Fig. 2) and a 76,000 M<sub>r</sub> protein in non-reducing conditions (data not shown). The anti-T8 and anti-T8A antibodies precipitated molecules of the same molecular weight (Fig. 2). Although these antigens appear similar and, in sequential precipitation studies anti-T8<sub>A</sub> largely removed both the T8A and T8 antigens, anti-T5 did not deplete T8 antigen (data not shown). These results suggest that the T5 and T8 antigens are not identical. A similar conclusion was reached after analysis of T-ALL tumour cells using anti T5 and anti-T8 in which some cell were reactive with anti-T8 but unreactive with anti-T5 (ref. 5).

Both the biochemical data and binding studies are consistent with the view that the T8 and T8<sub>A</sub> epitopes are located close together on a single molecule. However, proof of this conclusion must await peptide mapping and two-dimensional gel analysis. Nevertheless, the inhibition of CTL function implies that the epitope defined by anti-T8<sub>A</sub> may be critical for cytolytic function but not for suppressor function. Thus, these antigens seem to be homologous to Lyt2, which has been shown to inhibit murine CTL to varying degrees<sup>16,17</sup>. A recent report<sup>18</sup> suggests that another antibody defining the human cytotoxic cell population, termed anti-Leu2a, blocks CML. It is possible that this antibody may be directed against the same  $33,000-M_r$  protein.

The ability of anti-T8<sub>A</sub> to inhibit CML at the effector phase may involve blocking of structures near or associated with the antigen receptor of the T cell. Alternatively, Lyt2 or T8A may serve as molecules permitting attachment for conjugate formation between killer and target cells without themselves being antigen-specific receptors 19. Further experiments using purified and reconstituted T8A antigens will help to resolve these issues.

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# Anti-transferrin receptor monoclonal antibody and toxin-antibody conjugates affect growth of human tumour cells

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The development of the monoclonal antibody technique has led to renewed interest in whether the growth of tumour cells may be specifically inhibited by antibodies or antibody-toxin conjugates directed against antigenic determinants selectively expressed on tumour cells2-10. We have recently obtained monoclonal antibodies against the transferrin receptor of human cells<sup>11,12</sup>, which is thought to have an essential role in transport of Fe across the cell membrane and which is selectively expressed on proliferating cells in vivo and in vitro 13-17. In some cases, transferrin receptors can be used as a marker to distinguish between tumour cells and normal tissue 11,15-17. Here we show that human tumour cells are specifically killed in vitro by anti-transferrin receptor antibody covalently coupled to ricin or diphtheria toxic subunits. In experiments designed to test the effectiveness of these antibody-toxin conjugates in vivo, we found that anti-transferrin receptor antibody alone inhibits the growth of human melanoma cells in nude mice.

The mouse monoclonal IgG1  $\kappa$  antibody against the human transferrin receptor, designated B3/25, has been described previously<sup>11,12</sup>. The antibody was purified from ascitic fluid of tumour-bearing mice by precipitating with ammonium sulphate and fractionation on DEAE-cellulose. Ricin A toxic subunit was prepared from affinity-purified ricin toxin by reduction with 2-mercaptoethanol and fractionation on DEAE- and is. Diphtheria fragment A was prepared from CM-cellulose<sup>1</sup> diphtheria toxin (Connaught Laboratories) by tryptic cleavage<sup>1</sup> followed by reduction with 2-mercaptoethanol and chromatography on Sephacryl S-200. Antibody-toxin conjugates were N-succinimidyl-3-(2-pyridyldithio)synthesized using propionate (SPDP; Pharmacia)20. A sixfold molar excess of SPDP was used to introduce an average of three 2-pyridyldisulphide groups into the anti-receptor antibody. The modified antibody was then mixed with a threefold molar excess of freshly reduced ricin A subunit or diphtheria fragment A and incubated at 4°C for 36 h. The antibody conjugate was purified from unbound toxin subunits by chromatography on Sephacryl S-200. On average, 1-2 toxin subunits were covalently bound to each antibody molecule.

The effect of anti-transferrin receptor antibody-ricin A conjugate on protein synthesis in a human T leukaemic cell line, CCRF-CEM<sup>21</sup>, is shown in Fig. 1. It can be seen that the antibody-ricin A conjugates inhibit protein synthesis almost as effectively as the intact toxin. The residual protein synthesis in cells treated with high concentrations of the conjugate reflects the fact that the conjugate seems to act less rapidly than the intact toxin and is not observed if cells are exposed to the conjugate for 48 h. It is possible that cells differ in susceptibility to the antibody-ricin A conjugate depending on their position in the cell cycle. Antibody or ricin A subunit added alone or as a mixture, inhibited protein synthesis only at concentrations 200-1,000-fold higher than the antibody-ricin A conjugate. The specificity of the antibody-ricin A conjugates is further indicated by the fact that protein synthesis in a mouse lymphoma cell line, BW5147, was not inhibited by the anti-human transferrin receptor antibody-ricin A conjugate even though the cells were as sensitive as CCRF-CEM cells to the intact ricin toxin (Table 1). Specific inhibition of protein synthesis in CCRF-

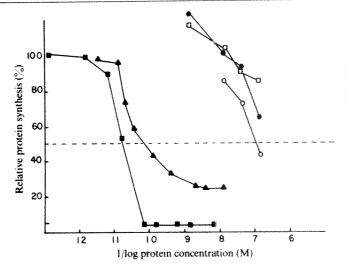


Fig. 1 Protein synthesis in a human leukaemic cell line is inhibited by anti-transferrin receptor-ricin A conjugate. CCRF-CEM cells (1×10<sup>6</sup>) were cultured for 20 h in 2.0 ml RPM1 1640 medium supplemented with 10% horse serum and various concentrations of ricin toxin (■), anti-transferrin receptor plus ricin A conjugate (Δ), ricin A subunit (Φ), anti-transferrin receptor antibody (□), or anti-transferring receptor antibody plus ricin A subunit (○). Then 10 μCi of <sup>3</sup>H-leucine (42 Ci mmol<sup>-1</sup>, NEN) were added to each dish and the cells were incubated for 2 h. Cells from duplicate cultures for each treatment were collected and incorporation of radioactivity into trichloroacetic acid-insoluble material was determined. Protein synthesis in treated cells was expressed as a percentage of <sup>3</sup>H-leucine incorporation into untreated control cells. Note that although ricin A was diluted in this experiment from a stock solution at 250 μg ml<sup>-1</sup> in 0.1% mercaptoethanol, in earlier experiments dialysis was carried out to remove the reducing agents without increasing the toxicity of the free ricin A chain.

CEM cells was also obtained with an anti-receptor antibody-diphtheria fragment A conjugate. Protein synthesis in HeLa cells and cells of a human melanoma cell line  $(M21)^{22}$ , was inhibited by anti-receptor antibody-ricin A, although M21 cells seemed less sensitive than CCRF-CEM cells.

As a further test of the ability of the anti-receptor antibodyricin A conjugate to kill tumour cells in vitro, and to assess the frequency with which cells might escape killing, we performed cloning assays. CCRF-CEM cells were plated out in twofold dilutions at concentrations ranging from  $5-1\times10^6$  cells per ml in 0.2 ml tissue culture medium in microtitue wells. No growth was observed even in wells containing  $2\times10^5$  cells in the presence of anti-receptor-ricin A antibody (10 nM), whereas a cloning efficiency of  $\sim30\%$  was observed in the absence of the con-

Table 1 Inhibition of protein synthesis in human tumour cell lines by anti-transferrin receptor-toxic subunit conjugates

Cell line	•	IC <sub>50</sub> Antibody-ricin A	Ricin toxin alone
CCRF-CEM human T leukaemia	1.5	0.06, 0.04, 0.09, 0.03*, 0.06*	0.03
M21 human melanoma	integration.	3.5	Suddistractions
HeLa	-	0.25	antimphoto
BW5147 mouse lymphoma	>11	> 13	0.04

The concentration (nM) required for 50% inhibition of protein synthesis (IC<sub>50</sub>) was calculated from results obtained in experiments of the type shown in Fig. 1. The same preparation of ricin A-antibody conjugate was used for all experiments, except for the two IC<sub>50</sub> values for CCRF-CEM cells marked with an asterisk: a separate preparation of conjugate was used in these two experiments.

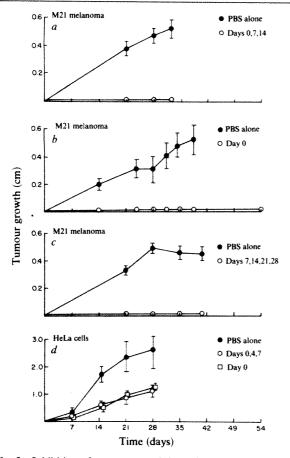


Fig. 2 Inhibition of tumour growth in nude mice by anti-transferrin receptor monoclonal antibody. Groups of BALB/c (nu/nu) mice (4 mice per group, females 4-5 weeks old) were given subcutaneous inoculations of 2×10<sup>7</sup> M21 melanoma or HeLa cells. Anti-transferrin receptor monoclonal antibody or antibodyricin A conjugate was then given i.v. in 0.2 ml phosphate-buffered saline (PBS) either immediately (day 0) or at the times indicated (open symbols). In a, 50 μg of ricin A-antibody conjugate was injected each time. For experiments b-d, 880 µg of unmodified antibody was given on each occasion. Control mice were injected with PBS alone (solid symbols). Tumour growth was estimated by measuring two perpendicular diameters of the tumours using calipers; results are expressed as geometric mean ± 1 s.e.

jugate. In similar experiments, no survivors were obtained when  $2 \times 10^5$  HeLa cells were treated with the same concentration of the antibody conjugate. In contrast, no effect was observed on the cloning efficiency of BW5147 mouse lymphoma cells

As the antibody-toxic subunit conjugates were effective in killing human tumour cells in vitro, we set out to determine whether the antibody-ricin A conjugates were effective in inhibiting the growth of human tumour cells in nude mice. As CCRF-CEM cells do not produce tumours in nude mice, M21 and HeLa cells were used. We found that the antibody-ricin A conjugate (50 µg intravenously (i.v.) on days 0, 7, 14) completely inhibited the growth of M21 cells in nude mice (Fig. 2a). However, a single i.v. injection of 880 µg of anti-transferrin receptor antibody alone also inhibited the growth of the melanoma cells at a subcutaneous site (Fig. 2b). The same results have been obtained in four similar experiments. In a fifth experiment, tumour formation was delayed but not completely inhibited. As shown in Fig. 2c, administration of antibody 7 days after inoculation of tumour cells was also effective in preventing tumour growth. However, the monoclonal anti-transferrin receptor antibody was much less effective in inhibiting growth of HeLa cells in nude mice even when repeated doses of antibody were given (Fig. 2d).

In another experiment, groups of mice were given a single i.v. injection at day 0 of either 10 or 50 µg of antibody or antibodyricin A conjugate. Mice given the higher dose of either unmodified antibody or conjugate did not develop tumours, whereas tumour growth in those mice receiving lower concentrations of each reagent was not significantly different from that of the controls. Thus, there is no evidence that the conjugate is more effective than unmodified antibody in inhibiting growth of the M21 melanoma cells in nude mice. However, this may reflect instability of the antibody-ricin A conjugate in vivo, or differences in the relative clearance of unmodified and conjugated antibody from the circulation, and requires further study. Furthermore, although the antibody-ricin A conjugate inhibits growth of HeLa cells in vitro, it is no more effective than antibody alone in preventing the growth of HeLa cell tumours in nude mice.

Recent reports have indicated that specific killing of tumour cells can be achieved in vitro with antibody-toxin conjugates<sup>2-5,7</sup>. We have shown here that similar results can be obtained with anti-human transferrin receptor antibodies coupled to ricin A subunit or diphtheria fragment A. More importantly, we have shown that growth of a human melanoma cell line in nude mice can be effectively inhibited by anti-human transferrin receptor antibody alone. In contrast, previous attempts to inhibit growth of murine lymphomas by administration of either anti-Thy-1 antibody<sup>5</sup> or ricin A-anti-Thy-1 antibody conjugates7 were only partially effective. Whether inhibition of M21 tumour growth is due to immunological mechanisms or to the effect of antibody on the ability of the tumour cells to compete for Fe in vivo remains to be determined. Clearly, however, sufficient antibody to inhibit growth can be delivered to the subcutaneous tumour by an i.v. injection. Furthermore, it seems that in vitro tests are of limited value in predicting the in vivo effects of antibody reagents on tumour growth.

Our preliminary results and the known properties of the transferrin receptor suggest that further studies of the usefulness of anti-receptor antibodies as therapeutic agents would be worthwhile. The transferrin receptor is a major glycoprotein on dividing tumour cells and is not rapidly shed from the cell surface (M. B. Omary and I. S. T., unpublished results). It is probably essential for cell growth, thus it is unlikely that tumour cells would be able to escape killing either by antigenic modulation<sup>23</sup> or by genetic loss of receptors<sup>24</sup>. As it is thought that receptor-bound transferrin is internalized<sup>25,26</sup>, the transferrin receptor might be expected to facilitate entry of bound antibody conjugates into the cell. Furthermore, anti-receptor antibodies may exert inhibitory effects on growth by interfering with Fe transport. Finally, as transferrin receptors are known to be present on mouse tumour cells, suitable animal models can be used to test the effectiveness of anti-receptor monoclonal antibody on in vivo growth of transplanted and spontaneous tumours and to examine the potential deleterious effects of antibody on normal dividing cells. A rat monoclonal antibody to the mouse transferrin receptor has recently been obtained (I.S.T., J. Lesley and R. Schulte, unpublished results).

Although the transferrin receptor is obviously not a tumourspecific antigen, there is little evidence that such antigens exist in man, and careful studies have always revealed that putative tumour-specific antigens are expressed on some normal cells<sup>27-29</sup>. Indeed, the transferrin receptor itself may be the putative cell surface glycoprotein associated with malignancy described elsewhere 30,31. Given this situation, it is probable that absolute specificity of anti-tumour antibody reagents is unlikely to be achieved. However, sufficient specificity may be obtained using monoclonal antibodies against target antigens such as the transferrin receptor, alone or in combination, to be of practical therapeutic importance.

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## Assignment of the genes for human λ immunoglobulin chains to chromosome 22

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Human immunoglobulin chains are expressed in somatic cell hybrids between mouse myeloma cells and human B cells<sup>1,2</sup>. By correlating the expression of human immunoglobulin chains and the presence of specific human chromosomes in the hybrid clones, we have assigned the human immunoglobulin heavychain gene cluster to human chromosome 14 (refs 1, 2). We have now studied somatic cell hybrids between mouse myeloma cells and either human peripheral lymphocytes or different human A chain-secreting lymphoblastoid cell lines. Somatic cell hybrid clones were pre-selected for their ability to produce human A immunoglobulin chains, and studied for the expression of isozyme markers assigned to each of the different human chromosomes. Subclones of human A chain-secreting hybrid clones were also studied. The results indicate that human chromosome 22 carries the human  $\lambda$  immunoglobulin chain genes.

We fused hypoxanthine phosphoribosyltransferase (HPRT)deficient P3×63Ag8 mouse myeloma cells<sup>3</sup> with either peripheral blood lymphocytes or different human lymphoblastoid cells (GM1056 that secretes IgA  $\alpha$ 2,  $\lambda$ ; GM923 that secretes IgA  $\alpha 1$ ,  $\lambda$ )<sup>4</sup> to map the chromosomal location of the human λ-chain genes. The hybrids were selected either in hypoxanthine-aminopterin-thymidine (HAT) medium or in HAT medium containing 10<sup>-4</sup> M ouabain<sup>1,2</sup>. After selection the hybrids were cloned in non-selective medium; each clone derived from an independent hybrid colony. As only one of the alleles for the different immunoglobulin chains is expressed in immunoglobulin-producing cells, the other being silent ('allelic exclusion')5, we can predict the presence of hybrids that have retained the chromosomes carrying immunoglobulin chain genes, but do not express them. In fact, we have shown previously that only a fraction of mouse × human hybridomas containing human chromosome 14 expresses human heavy chains<sup>1,2</sup>. Recently, by using  $\mu$  chain-specific cDNA probes<sup>4</sup> and Southern blotting procedures<sup>6</sup>, we have confirmed assignment



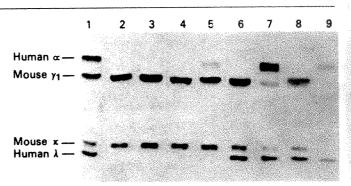


Fig. 1 SDS-polyacrylamide gel electrophoresis of immunoglobulin chains secreted by somatic cell hybrids between P3 × 63Ag8 mouse myeloma cells<sup>3</sup> and GM1056 human lymphoblastoid cells<sup>4</sup>. Parent and hybrid cells were grown for 24 h in leucine-deficient media containing 100 μCi ml<sup>-1</sup> <sup>3</sup>H-leucine (70 Ci mmol<sup>-1</sup>). Cells were pelleted and supernatants (500 μl) reacted with 25 μl of rabbit anti-human λ chain-specific sera and rabbit anti-mouse immunoglobulins for 1 h in ice. 100 µl of a 10% suspension of fixed Staphylococcus aureus 1.2 were added to each reaction mixture for 30 min, or 50  $\mu$ l of goat anti-rabbit  $\gamma$ -globulin sera were added for 16–18 h, at 4°C. Double antibody precipitates were collected by centrifugation through a pad of 0.2 ml 1 M sucrose. All immuse precipitates were washed with 5% sucrose, 15 mM Tris-HCl pH 7.4, 0.5 M NaCl, 5 mM EDTA, 1% NP-40, 2 mM phenylmethylsulphonyl fluoride. The immune precipitates were resuspended in Laemmli buffer17 and analysed on 10-11% polyacrylamide gels<sup>17</sup>, embedded in 2,5-diphenyloxazole and subjected to fluorography<sup>18</sup> for 5-14 days. Lanes 2 and 9 show the immunoprecipitates of the culture fluids of P3×63Ag8 and GM1056 respectively. GM1056 cells secrete IgA ( $\alpha 2$ ,  $\lambda$ ). The  $\lambda$  chain produced by these cells migrates faster than the mouse  $\kappa$  and the human  $\lambda$  chains produced by independent hybrid clones between P3  $\times$  63Ag8 cells and human peripheral lymphocytes (see Figs 2, 3). In lanes 1, 3-8 are the immunoprecipitates of P3  $\times$  63Ag8  $\times$  GM1056 hybrids: human  $\alpha$  and  $\lambda$  chains segregate independently in these hybrids. Immunoprecipitation of cytoplasmic immunoglobulin chains 1.2 present in the hybrids was also carried out, with identical results (data not shown).

of the human heavy-chain genes to human chromosome 14 (T. Dolby, A. Dayton and C. M. C., in preparation). Because of allelic exclusion<sup>5</sup> we decided to pre-select mouse×human hybridomas for the expression of human  $\lambda$  chains (Figs 1, 2) and to study the  $\lambda$  chain-producing hybrids for the expression of isozyme markers assigned to each of the different human chromosomes. The independent hybrid clones segregated into  $\lambda$ chain-secreting and non-secreting hybrids (Fig. 1). As the parental mouse myeloma cells were secreting immunoglobulins. each of the hybrids producing a human immunoglobulin chain was also secreting it1,2.

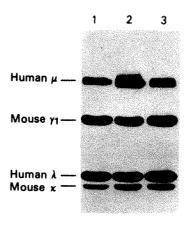


Fig. 2 SDS-polyacrylamide gel electrophoresis of immunoglobulin chains secreted by somatic cell hybrids between P3× 63Ag8 mouse myeloma cells<sup>3</sup> and human peripheral blood lymphocytes. The human and mouse immunoglobulin chains were labelled and immunoprecipitated as described in Fig. 1 legend. The independent hybrids (lanes 1-3), pre-selected for isozyme analysis, were expressing human A chain. Immunoprecipitation of cytoplasmic immunoglobulin chains present in the hybrids was also carried out, with identical results (data not shown).

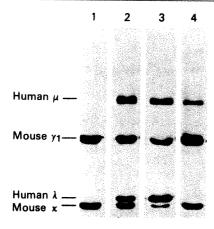


Fig. 3 SDS-polyacrylamide gel electrophoresis of immunoglobulin chains secreted by four hybrid subclones of a hybrid clone between P3×63Ag8 cells and human peripheral blood lymphocytes (see Fig. 2). The expression of the human  $\mu$  and  $\lambda$  chains segregated independently in the hybrid subclones. The subclones in lane 1 have lost the ability to secrete (produce) human  $\mu$  and  $\lambda$ chains, whereas those in lanes 2 and 3 have retained the expression of these chains. The subclones in lane 4 have lost the ability to secrete (produce) human  $\lambda$  chains. Immunoprecipitation of cytoplasmic immunoglobulin chains present in the hybrids was also carried out, with identical results (data not shown).

Table 1 shows that only human chromosomes 14 and 22 are likely to carry the human  $\lambda$  heavy-chain genes, as hybrid clones lacking each one of the other human chromosomes were found to produce human  $\lambda$  chains. As the genes for heavy,  $\kappa$  and  $\lambda$  immunoglobulin chains lie on different chromosomes 1.2.7.8, and

Table 1 Human chromosomes present in mouse-human hybridomas producing human immunoglobulin λ chains

	No. of hybridoma clones that are:		
Human chromosome	+/+	+/	
1	0	11	
2 3	0	11	
3	5	7	
4	6	6	
4 5 6	9	3	
6	10	10	
7	3	9	
8	6	14	
9	0	12	
10	4	8	
11	8	4	
12	2 3	10	
13	3	9	
14	20	0	
15	9	3	
16	2	10	
17	18	2	
18	5	6	
19	1	11	
20	4	8	
21	2	10	
22	14	0	
X	18	2	

Hybrid cells were studied for the expression of isozyme markers assigned to each of the different human chromosomes by starch gel or cellulose acetate gel electrophoresis: chromosomes 1, enolase 1 (EC 4.2.1.11); 2, isocitrate dehydrogenase (EC 1.1.1.42); 3,  $\beta$ -galactosidase (EC 3.2.1.23); 4, phosphoglucomutase 2 (EC 2.7.5.1); 5, hexosaminidase B (EC 3.2.1.30); 6, glyoxalase-1 (EC 4.4.1.5) and phosphoglucomutase 3 (EC 2.7.5.1); 7,  $\beta$ -glucuronidase (EC 3.2.1.31); 8, glutathione reductase (EC 1.6.4.2); 9, aconitase (EC 4.2.1.3); 10, glutamate oxaloacetic transaminase (EC 2.6.1.1); 11, lactate dehydrogenase A (EC 1.1.1.27); 12, lactate dehydrogenase B (EC 1.1.1.27); 13, esterase D (EC 3.1.1.1); 14, nucleoside phosphorylase (EC 2.4.2.1); 15, mannosephosphate isomerase (EC 5.3.1.8); 16, adenine phosphoribosyltransferase (EC 2.4.2.7); 17, galactokinase (EC 2.7.1.6); 18, peptidase A (EC 3.4.11.); 19, glucose phosphate isomerase (EC 5.3.1.9); 20, adenosine deaminase (EC 3.5.4.4); 21, superoxide dismutase 1 (EC 1.15.1.1); 22, arylsulphatase (EC 3.1.6.1); X chromosome, glucose-6-phosphate dehydrogenase (EC 1.1.1.49). +/+, Clones that both produce human λ chains and do not carry the numbered chromosome.

**Table 2** Expression of human  $\lambda$  chains in mouse  $\times$  human hybridoma subclones

	No. of hybridoma subclones that are:			
Human chromosome	+/+	+/-	-/+	-/-
1	0	9	0	7
2	0	9	0	7
3	8	5	4	3
4	4	5	3	4
5	3	6	5	2
6	16	2	6	1
7	0	9	2	5
8	0	9	0	7
9	0	10	0	7
10	0	9	0	7
11	5	4	6	1
12	2	7	2	5
13	1	8	1	6
14	9	2	6	1
15	3	6	4	3
16	0	9	0	7
17	13	1	6	1
18	2	7	2	5
19	0	9	0	7
20	1	8	1	6
21	0	8	0	8
22	7	0	0	7
X	5	2	6	1

+/+, +/-, Same as for Table 1; -/+, clones that do not express human  $\lambda$  chains and carry the numbered chromosome; -/-, clones that neither express human  $\lambda$  chains nor carry the numbered chromosome.

as the heavy-chain genes are located on human chromosome 14 (refs 1, 2), human chromosome 22 is the likely candidate for carrying the human λ-chain genes. All 20 independent hybrid clones tested for nucleoside phosphorylase (a marker of human chromosome 14) were positive for the enzyme as this chromosome is preferentially retained in mouse × human hybridomas<sup>9</sup>. To exclude the possibility that the human  $\lambda$ -chain genes are located on human chromosome 14 and to confirm their assignment to human chromosome 22, we subcloned five independent  $\lambda$  chain-secreting (producing) clones, and studied them for expression of human  $\lambda$  chains (Fig. 3) and of isozyme markers assigned to each of the different human chromosomes. As shown in Table 2, chromosome 14 can be excluded from consideration as two subclones have lost chromosome 14 yet have retained the ability to produce human  $\lambda$  chains. In addition, the results confirm that the genes for  $\lambda$  chains are on human chromosome 22, as this is the only human chromosome consistently present in all subclones secreting human  $\lambda$  chains (Table 2, 2nd column). Loss of human chromosome 22 results in loss of the ability to produce human  $\lambda$  chains (Table 2, 4th column and Fig. 3, lane 4).

Our results parallel those obtained by Southern blotting analysis of hybrid cell DNAs using nucleic acid hybridization probes (W. McBride and P. Leder, personal communication). These findings, in conjunction with the assignment of the heavychain gene clusters to human chromosome 14 (refs 1, 2) and of the human k-chain gene to human chromosome 2 (W. McBride and P. Leder, personal communication), suggest a possible relationship between specific chromosome translocations, immunoglobulin genes and the expression of malignancy in Burkitt's lymphoma<sup>10</sup>. A translocation between chromosomes 8 and 14, described as t (8, 14) (q24; q32), has been found in numerous cases of the disease<sup>11,12</sup>. Recently, two different translocations involving human chromosome 8 have been described in non-African Burkitt's lymphoma13-15, which involve the same segment of human chromosome 8 and either human chromosome 22 (that carries the  $\lambda$ -chain genes) or chromosome 2 (that carries the  $\kappa$ -chain gene). More recently, similar translocations, t (2, 8) (p12; q24) and t (8, 22) (q24; q11), have also been found in cases of African Burkitt's lymphoma<sup>1</sup> These findings indicate that approximately the same segment of human chromosome 8 is translocated to each of the human chromosomes carrying immunoglobulin genes in Burkitt's lymphoma. It would be interesting to determine whether the

break points on human chromosomes 2, 14 and 22 affect chromosomal DNA segments that lie close to or contain the human immunoglobulin chain genes.

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# Phaseolin mRNA is translated to vield glycosylated polypeptides in Xenopus oocytes

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The cotyledons of developing bean (Phaseolus vulgaris L.) seeds rapidly synthesize and accumulate the storage globulin, phaseolin1, which seems to be encoded as a small multigene family<sup>2,3</sup>. Phaseolin from the cultivar 'Tendergreen' has been resolved electrophoretically into three components (of differing molecular weights), designated  $\alpha$  (51,000),  $\beta$  (48,000) and  $\gamma$ (45,500). The in vitro synthesis of phaseolin has been investigated previously using a cell-free translation system derived from wheat germ<sup>4</sup>. The *in vitro* products did not co-migrate with the authentic polypeptides, the differences apparently being due to the lack of glycosylation of the primary transcripts. We have now used Xenopus oocytes to translate mRNA from bean cotyledons and find that phaseolin is synthesized in a glycosylated form very similar to authentic phaseolin and is transported into the membrane fraction of the cells.

Translation of bean cotyledon mRNA in cell-free extracts from wheat germ4 yielded polypeptide products that were immunoprecipitated by phaseolin-specific antibodies and which gave peptide maps, using the Cleveland procedure5, similar to those of authentic protein<sup>6</sup>. However, SDS-polyacrylamide gel electrophoresis (PAGE) of the in vitro products resolved only two bands that migrated close to, but slightly faster than, the positions of the  $\beta$  and  $\gamma$  bands of the native protein<sup>4</sup>. It has previously been suggested that these two bands represent unglycosylated forms of phaseolin, the lower band consisting of co-migrating, unprocessed forms of the  $\beta$  and  $\gamma$  polypeptides and the upper band consisting of unprocessed  $\alpha$  polypeptides. This hypothesis is consistent with the different degrees of glycosylation of the  $\alpha$ ,  $\beta$  and  $\gamma$  polypeptides (3:3:1 ratio of sugar content respectively)7.

We have now investigated the hypothesis further by injecting phaseolin mRNA into the cytoplasm of Xenopus laevis oocytes and then analysing the translation products of the phaseolin mRNA by one-dimensional SDS-PAGE (Fig. 1). Three major polypeptides were immunoprecipitated with antibody prepared against phaseolin and these migrated at positions closely approximating those of authentic phaseolin (Fig. 1, lane c). In some preparations (Fig. 1, lane k), the  $\beta$  component separated into a closely migrating doublet, possibly representing slight differences in the processing of these polypeptides. The smallest component of the oocyte products migrates with a higher molecular weight (46,500) than that of the authentic protein (45,500). When the glycosylation inhibitor tunicamycin<sup>8</sup> was co-injected with phaseolin mRNA (Fig. 1, lane h), only two immunoprecipitable products were resolved. These co-migrate with the polypeptides synthesized in vitro by wheat-germ extracts in the presence of phaseolin mRNA (Fig. 1, lane a). This effect was seen most clearly when glycosylation intermediates (dolichol phosphate) were depleted by incubation of

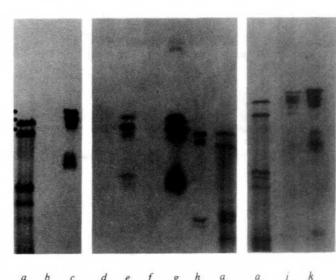
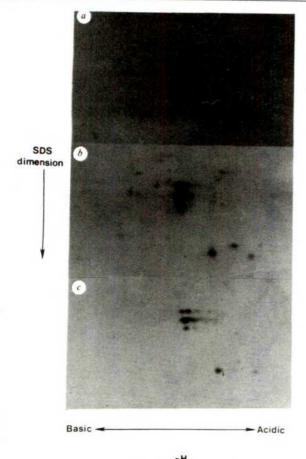


Fig. 1 Oocytes were removed from the toad and maintained in modified Barth's solution 15 at 19 °C. Phaseolin mRNA was isolated and translated in the wheat-germ cell-free system4. Phaseolin mRNA (20 ng) was injected into isolated oocytes. To study glycosylation inhibition, tunicamycin (40  $\mu g \, ml^{-1}$ ) was co-injected with the mRNA, and for such experiments, tunicamycin  $(2 \mu g \text{ ml}^{-1})$  was included in the culture medium. Unless otherwise stated, after 24 h incubation, the oocytes were placed in medium containing 2 mCi ml<sup>-1</sup> <sup>3</sup>H-leucine for a further 24 h. The radiolabelled oocytes were homogenized in 20 mM sodium phosphate pH 7.2, containing 0.5 M NaCl, 1% Nonidet P40 and 1 mM phenylmethylsulphonyl fluoride (40 µl per oocyte). Yolk and pigment were pelleted for 2 min in a microfuge. The homogenate was either analysed directly by SDS-PAGE or immunoprecipitated as described by Sun *et al.*<sup>1</sup>. For subcellular localization studies, oocytes were treated as described elsewhere<sup>8</sup>. The figure shows fluorographs<sup>16</sup> of products separated by SDS-PAGE. The lanes represent: a, total products synthesized in wheat germ in response to phaseolin mRNA; b, immunoprecipitate of mockinjected oocytes; c and k, immunoprecipitate of oocytes injected with phaseolin mRNA; d, immunoprecipitate of the cytosol fraction of oocytes injected with buffer only; e, immunoprecipitate of the cytosol fraction of oocytes injected with phaseolin mRNA; f, as d, membrane fraction of an equivalent amount of oocytes; g, as e, membrane fraction of an equivalent amount of oocytes; h, immunoprecipitate of oocytes co-injected with phaseolin mRNA and tunicamycin and placed in medium containing radioactive amino acids after 24 h incubation; and j, as h, except that injected oocytes were placed immediately in radiolabelled medium. Represents the position of stained authentic phaseolin run in the adjacent lane. The specificity of the immunoprecipitation reaction has been demonstrated previously, using the translation products of brome mosaic virus RNA as a control1

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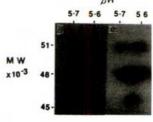


Fig. 2 Oocyte products, prepared as described in Fig. 1 legend, were separated by two-dimensional gel electrophoresis9. a Shows the separation of labelled proteins from mock-injected oocytes; b shows a similar separation of proteins from oocytes injected with phaseolin mRNA. The proteins in c are immunoprecipitates of those in b and are shown, enlarged, in e. d Shows a two-dimensional separation of authentic phaseolin of the cultivar 'Tendergreen'.

the injected oocytes for 24 h before the addition of radioactive amino acids to the medium (Fig. 1, lane h). However, when oocytes injected with phaseolin mRNA and tunicamycin were immediately placed in medium containing 3H-leucine, both glycosylated and unglycosylated forms of phaseolin were resolved (Fig. 1, lane j).

Two-dimensional separation<sup>9,10</sup> of phaseolin polypeptides synthesized in oocytes revealed that they co-migrated precisely with authentic protein in the isoelectric focusing dimension, the α component separating into two charge isomers. In the SDS-PAGE dimension, the y component migrates slightly more slowly than the authentic component (Fig. 2). The lower molecular weight components visible in the immunoprecipitate are probably the globulin-2/albumin proteins of P. vulgaris<sup>11</sup>. The mRNA preparation codes for these proteins in addition to phaseolin and the antiserum shows some reactivity with them. The identity of the minor high molecular weight products visible in the immunoprecipitates is unknown.

Phaseolin is transported across the endoplasmic reticulum of bean cotyledon cells before its accumulation in the protein

storage bodies1,12. Comparison of polypeptides accumulating in the cytosol (Fig. 1, lane e) and membrane fractions (Fig. 1, lane g) of the oocytes revealed that the bulk of phaseolin was localized in the membrane fraction. The polypeptides present in the cytosol may represent contamination during the isolation procedure; glycosylation probably occurs in the cytosol8.

Therefore, phaseolin is synthesized in oocytes as a glycosylated form which virtually co-migrates with authentic phaseolin. The slight difference in size of the  $\gamma$  band may be explained by the fact that, although oocytes can glycosylate primary translation products at the correct site<sup>13</sup>, the attached sugar moiety may not be identical with that attached in the bean cell. Phaseolin is associated with the membrane fraction of oocytes. The storage proteins of corn (zein) have also been synthesized in oocytes in membrane fractions reminiscent of protein bodies14 Such observations suggest that these proteins carry signals intrinsic to the formation of the storage organelles characteristic of many proteinacious seeds. Zein is not glycosylated but is processed by cleavage of a signal sequence 14. The results reported here argue against the involvement of signal sequence cleavage during the processing of phaseolin because the products synthesized in the presence of tunicamycin, which inhibits glycosylation but does not interfere with sequence cleavage, are the same size as those synthesized in wheat germ. Xenopus oocytes are, therefore, a valuable tool with which to investigate the processing of phaseolin.

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# An 'internal' signal sequence directs secretion and processing of proinsulin in bacteria

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Most secreted proteins, both eukaryotic and prokaryotic, contain an amino-terminal extension that is removed some time during transport (see ref. 1 for a review). The signal states that these amino-terminal extensions (presequences or signal sequences) serve to bind the protein to the membrane and then to lead the protein through. We recently constructed a series of plasmids in which parts of the Escherichia coli prepenicillinase signal sequence4 were contiguous with the gene for rat preproinsulin, containing 21 of the 24 codons that code for the preproinsulin signal sequence. When Escherichia coli was transformed with each of these hybrid signal sequence constructions, an insulin antigen was secreted5 and correctly

processed to proinsulin6 in every case. One of these coustructions fused the first half of the prepenicillinase signal sequence codons to the preproinsulin gene; as insulin antigen was not secreted when the same codons were fused to the DNA coding only for proinsulin and hence lacking the eukaryotic signal sequence, we hypothesized that the enkaryotic signal sequence was sufficient to direct transport in bacteria. We could not, however, eliminate some minor but specific role for the amino terminus donated by the bacterial signal sequence. One way to do this is to replace the prepenicillinase sequences with that of a nonsecreted E, coll protein, for example  $\beta$ -galactosidase. We show here that E. coll harbouring a plasmid encoding a  $\beta$ -galactosidase-preproinsulin fusion protein efficiently secretes insulin antigen and processes the protein to proinsulin despite the fact that the signal sequence is internal to the amino terminus.

The plasmid pExlac1507 is a derivative of pBR3228 with a lac fragment inserted such that unique EcoRI and HindIII sites directly follow the first eight codons of  $\beta$ -galactosidase and the lac UV5 promoter. A PstI-terminated cDNA copy of the gene for rat preproinsulin (lacking the first three preproinsulin codons) was inserted into the *HindIII* site of pExlac150 by first removing the 3'-end overhang with the Klenow fragment of E. coli DNA polymerase I, then ligating HindIII linkers to the blunt ends, cutting with HindIII and ligating the fragment to HindIII-cut pExlac150. Figure 1 shows the DNA sequence across the region of interest on this construction, called plac 1947, from the fMet codon of  $\beta$ -galactoridase to the fourth codon of the preproinsulin gene insert (which is the seventh codon of the true preproinsulin gene). There are 18 non-signal sequence amino acids at the amino terminus before the first signal sequence amino acid: 8 are encoded by the first 8 codons of the  $\beta$ -galactosidase gene, 5 by the inserted EcoRI-HindIIIlinker, and 5 by the poly-GC tails of the cDNA insert

plac1947 transformed into E. coli K-12 strain PR13 was grown in sulphur-deficient medium, with two control constructions in the same E. coli strain. These were p241.1947, which encodes preproinsulin and where more than 90% of the insulin antigen is secreted into the periplasmic space, and p241.CB15, which lacks a signal sequence hydrophobic core and where more than 90% of the insulin antigen remains inside the cell, as determined by radioimmunoassay and reported in detail elsewhere<sup>5</sup>. Figure 1 shows the amino acid sequences of the various encoded fusion proteins of plac1947, p241.1947 and p241.CB15 and rat preproinsulin, for comparison. Equal

amounts of late log cells were labelled with 35SO42- and either lysed with lysozyme and Triton X-100 to produce a whole cell lysate or digested with lysozyme in sucrose to release the contents of the periplasmic space. Immunoprecipitated insulin antigen was electrophoresed on a 7-15% gradient Laemmli<sup>10</sup> SDS-polyacrylamide gel. Figure 2 shows an autoradiograph of the gel; the three whole-cell samples are on the left, the three periplasmic samples are on the right. Only 9% of the insulin antigen of the non-secreting control, p241.CB15 (whole cell lysate, lane 1; periplasmic contents, lane 6), is released during lysozyme-EDTA digestion, as determined by densitometer tracings; thus, less than 10% of the cells lyse during the treatment to release the contents of the periplasm. Both the new construction, plac 1947 (whole cell lysate, lane 2; periplasmic content, lane 5), and the control, p241.1947 (whole cell lysate, lane 3; periplasmic content, lane 4), efficiently secrete insulin antigen into the periplasm. Moreover, the  $\beta$ -galactosidasepreproinsulin fusion protein seems to be correctly processed to proinsulin, as it co-migrates in a tight band with the immunoprecipitated protein product of p241.1947, which has been sequenced to prove that it is correctly cleaved to proinsulin6. The protein product of p241.CB15, which is not secreted, is 22 amino acids longer<sup>5</sup> than proinsulin and migrates above the proinsulin band. These data are consistent with the hypothesis that the protein product of the  $\beta$ -galactosidase-preproinsulin gene construction is also properly processed to proinsulin, although a difference of one or two amino acids cannot be ruled out.

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Our data show that a cukaryotic signal sequence, preceded by 18 amino acids at its amino terminus arising from DNA sequences totally unrelated to any signal peptide, can still direct efficient secretion and also probably correct clipping. Lingappa et al. have proposed that ovalbumin contains an internal signal sequence; our experiment supports the concept that the hydrophobic core need not be immediately at the amino terminus to function, although there are limits to the functional placement of the signal sequence. Bedoulle et al. 12 have used genetic techniques to fuse most of the gene for a bacterial periplasmic protein, the maltose binding protein, to most of the gene for  $\beta$ -galactosidase.  $\beta$ -Galactosidase enzymatic activity was not detected in the periplasm, but was present in the inner membrane and cytoplasmic fractions<sup>12</sup>. Thus, the instructions for secretion can be overruled if other regions of the protein obscure the presence of a signal sequence or, once secretion is initiated, inhibit completion of secretion.

Fig. 1 Comparison of the DNA sequence across the fusion region in placi 1947 and amino acid sequence of the encoded hybrid  $\beta$ -galactosidate-preproissulin protein product with the amino acid sequences of the three hybrid prepenicillinase-prepromisilin protein products<sup>3</sup>. The DNA sequence begins at the fMet codon of  $\beta$ -galactosidase, contributed by pExlac 150, and ends at the seventh codon of the pre-proinsulin signal sequence, contributed

	MetThrMetIleThrAspSerLeuGluPheGlnAlaTrpGlyGlyGlyGlyGlyTrpMetArgPhe
P/ac 1947	ATGACCATGATTACGGATTCACTGGAATTCCAAGCTTGGGGGGGG

p/ac1947	MTMITDSL		EFQAVGGGG	G WERFLPLLALLVLWEPEPAQA	PVKQ
p241.1947	*	MSIQHFRVALIP	LQGGGGG	<b>VMRFLPLLALLVLVRPKPAQA</b>	FVKQ
p241.CB15		MSIQHFRVALIP	LQR	EPKPAQA	FVKQ
Preproinculin				MALVMRPLPLLALLVLVEPKPAQA	FVKQ

by a HindIII-terminated cDNA meert encoding rat prepromsulin, with the intervening codons (underlined) contributed by an EcoRI-HindIII linker and the poly-GC tails of the preproinsulin insert. Each line of amino acid sequence represents one continuous sequence which has been grouped from left to right to emphasize similarities and differences as follows: first group, β-galactosidase amino acids; second group, preproinsulin as amino acids; third group, amino acids encoded by DNA linkers and the poly-GC tails of certain preproinsulin gene inserts; fourth group, preproinsulin as an acids; fifth group, proinsulin amino acids; through the fourth amino acid. The protein product of p241.1947 is effectively secreted by bacteria, where the protein product of p241.E15 is not secreted at all? A = Aia, C = Cys, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, K = Lys, L = Leu, M = Met, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, V = Val, W = Trp plac1947 was constructed as follows: 0.2 μg of the Print-terminated cDNA insert from p241.1947, encoding the gene for preproinsulin (but minus the first three codons) and solated by the methods described elsewhere<sup>13</sup>, was incubated with 1.4 units of E. coli DNA polymerase I (Klenow fragment, New England Biolabs) for 2 h at 37 °C in 50 μ N 10 mM Tris-HCl pH 7.5, 10 mM MgCl<sub>22</sub>. 1 mM dithicthretiol (DTT), 50 mM NaCl, 0.1 mM dCTP. The mixture was phenol extracted, ethanol precipitated and spun in a deak-top clinical centrifuge for 2 min through 2 ml Sephadex G-50 (Pharmacia) in 20 mM NaCl. The ethanol precipitation was resuspended in 10 μ 150 mM Tris-HCl pH 7.6, 10 mM MgCl<sub>22</sub>, 1 mM DTT, 1 mM ATP, and incubated with 0.2 μg of kinased HindIII linker (Collaborative Research) and 15 units of T4 DNA lagase (New England Biolabs) overnight at 15 °C. After phenol extraction and ethanol precipitation, the fragment was subcloned into HindIII (New England Biolabs) for 4 h at 37 °C in 100 μl 10 mM Tris pH 7.6, 10 mM MgCl<sub>23</sub> 1 mM DTT and 50 mM NaCl. The cut fragment was subcloned into Hi

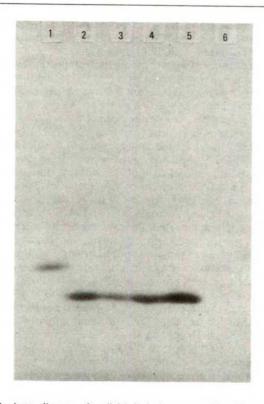


Fig. 2 Autoradiogram of radiolabelled, immunoprecipitated protein products of three preproinsulin constructions in E. coli K-12 strain PR13 fractionated for whole cell contents or for the contents of the periplasm. Lane 1, p241.CB15, whole cell; lane 2, plac1947, whole cell; lane 3, p241.1947, whole cell; lane 4, p241.1947, periplasm; lane 5, plac1947, periplasm; lane 6, p241.CB15, periplasm. Cells bearing the preproinsulin plasmids were grown in  $2YT^{17}$  overnight.  $100 \,\mu$ l were inoculated into  $10 \,\mathrm{ml} \,\mathrm{S}$  medium<sup>18</sup> supplemented with  $10 \,\mu\mathrm{g} \,\mathrm{ml}^{-1}$  thiamine,  $40 \,\mu\mathrm{g} \,\mathrm{ml}^{-1}$  L-threonine, and  $40 \,\mu\mathrm{g} \,\mathrm{ml}^{-1}$  L-leucine, and grown to an  $A_{550}$  of 0.7–0.85 at  $37 \,^{\circ}\mathrm{C}$ .  $5 \,\mathrm{mCi} \,\mathrm{H}_2^{35}\mathrm{SO}_4$  (carrier-free, NEN) were added to the cells, which were allowed to grown for a further  $10 \,\mathrm{min}$  then collected and superproduction. were allowed to grow for a further 10 min, then collected and resuspended in  $400 \mu l$  50 mM Tris-HCl pH 8, 25% sucrose. The cells were divided into two 200- $\mu l$  aliquots which were both incubated on ice for 15 min with 50  $\mu l$ lysozyme in 20 mM EDTA pH 8. A whole-cell fraction was prepared from one of the 200-µl aliquots by lysing the cells with 750 µl 150 mM Tris-HCl pH8, 0.2 M EDTA, 2% Triton X-100, and pelleting the cell debris by centrifugation for 1 h at 16,500 r.p.m. in a Sorvall SA-600 rotor. A periplasmic fraction was prepared from the other 200-µl aliquot by centrifugation for 20 min at 8,000 r.p.m. in a Sorvall SS-35 rotor and dilution of the supernatant with 750  $\mu$ l of the Triton buffer described above. Both fractions were incubated for 1 h at 37 °C with an amount of anti-insulin serum (an IgG fraction), that is in a 1,000-fold excess over the insulin antigen level in p241.1947 (determined by radioimmunoassay5). 100 µl of heat killed, formalin-treated Staphylococcus aureus (10% v/v, prepared by the method of Kessler<sup>19</sup>) was added, incubated on ice for 30 min and washed by the method of Kessler<sup>19</sup>. The *S. aureus*-radiolabelled antigen complexes were prepared as previously described<sup>6</sup>, except that the samples were resuspended in  $50 \,\mu l$  sample buffer and electrophoresed on a 7-15% polyacrylamide gradient gel using Laemmli buffers10 supplemented with 2 mM EDTA. The cells harbouring plac 1947, p241.1947 or p241.CB15 were grown to  $A_{550}$  0.84, 0.76 and 0.74, respectively. Equal whole-cell and periplasmic samples were loaded as follows: plac 1947, 1 µl; p241.1947, 1 µl; p241.CB15, 2.5 µl. The gel was destained, dried and autoradiographed for 6.h on Kodak X-omat AR-5 film. The film was scanned with an Ortec 4310 densitometer. The actual gel was 20 cm, but only a 3-cm portion is shown here; no other bands appeared in the lanes. By standard liquid radioimmunoassay, described elsewhere<sup>5</sup>, plac 1947 makes ~1,500 molecules per cell in PR13 (I. Akerblom, personal communication), a whole-cell insulin antigen level similar to that of p241.19475. All manipulations involving cells bearing preproinsulin plasmids were done under P1 containment according to NIH guidelines.

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# Point mutation in the TATA box curtails expression of sea urchin H2A histone gene in vivo

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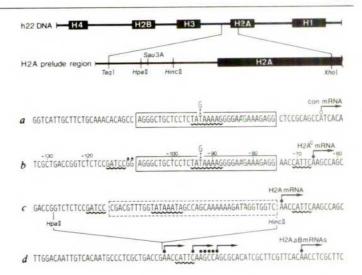
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In most, but not all, eukaryotic genes transcribed by polymerase B (II)1-5, the site of initiation of transcription is preceded by a TATATAT sequence4, commonly referred to as the Goldberg-Hogness<sup>6</sup> or TATA box, which seems to be required for the generation of faithful 5' termini of mRNAs<sup>7-14</sup>. For all genes except one<sup>9,12,15-17</sup>, deletion of the TATA box reduces the rate of mRNA synthesis<sup>7,10,11,13,18-23</sup>. Point mutations in the TATA box of the chick conalbumin gene diminish transcription in vitro 20-40-fold<sup>21,24</sup>. The question remained of whether this effect would persist in vivo where far upstream sequences dramatically modulate gene expression 7-11,25,26. Unfortunately the chicken conalbumin gene is poorly expressed when introduced into eukaryotic cells in culture (B.W., unpublished results) or into Xenopus oocytes (S. Swany, personal communication). We have therefore constructed a new gene unit with a chimaeric promoter region by introducing the conalbumin wild-type or mutated TATA box region into the sea urchin H2A histone gene, which is known to be efficiently expressed in Xenopus oocytes<sup>27-29</sup>. We report here that with both the wild-type and mutated chimaeric promoters, initiation of transcription occurs at a novel initiation site about 26 nucleotides downstream from the TATA or TAGA boxes, but in the mutant case, the amount of specific transcripts is decreased fivefold.

The scheme for construction of the chimaeric promoter was based on restriction maps and sequencing data of the conalbumin<sup>30</sup> and the H2A histone genes<sup>7,31</sup> (see Fig. 1 legend). This enabled us to assemble a novel gene with a chimaeric promoter which we called H2Ac. The TATA- and the TAGA-H2Ac genes were subsequently reintroduced into the 6-kilobase (kb) histone DNA repeat unit, in the place of the wild-type H2A gene. Successful construction of the chimaeras was confirmed at this stage by DNA sequencing<sup>32</sup>. Our sequence manipulations leave the upstream modulator sequences8 and the initiator

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Fig. 1 Structure of the 5'-flanking region of the H2A wild-type and mutant genes in the context of the 6-kb histone gene repeat unit. The structural maps of the exchanged DNA segments are shown in the context of the wild-type sequences remaining. A-D Depict the nucleotide sequence of the TATA/TAGA region of the chicken conalbumin, the chimaeric TATA/TAGA H2Ac, the wild-type H2A and the TATA box deletion H2A genes respectively. The exchanged DNA segments are indicated by boxes. The chimaeric promoter mutants contain two extra nucleotides originating from a BamHI linker (A). Conserved sequences underlined. ●, Map positions of the various H2A mRNA 5' ends (see Fig. 3); ○, the conalbumin mRNA 5' end<sup>30</sup>. The numbering of the TATA-H2Ac gene refers to the initiation codon of the gene as +1. The chimaeric H2A histone/conalbumin gene promoter mutants were constructed as follows. To replace the TATA box region of the H2A histone gene with the equivalent fragment of the chicken conalbumin gene, we first constructed pBR322-TATA and -TAGA recombinants. Short fragments containing these sequences were prepared from the appropriate mutant and wild-type conalbumin DNAs<sup>21,24</sup> by digestion with BstNI (-44) and MboII (-9). The ends of each fragment were flush-ended with T4 DNA polymerase. After heat inactivation, <sup>32</sup>P-labelled BamHI



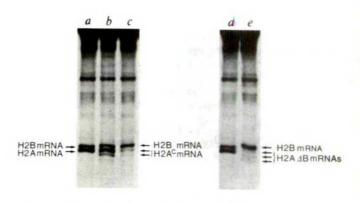
linkers were ligated to the flush ends. After BamHI restriction, the DNA fragments terminating in Bam sequences were purified and cloned into the BamHI site of pBR322. After amplification, the cloned TATA- and TAGA-containing BamHI fragments were recovered and treated with HaeIII enzyme to remove the Bam-linker sequence at the 3' end of the coding strand. The resulting fragments were ligated to the HincII-Xho fragment of the H2A gene<sup>7</sup> and the appropriate product selected by gel electrophoresis. The selected DNA fragments were ligated to the 90-bp EcoRI-Sau3A fragment of the pBR H2A-3 DNA<sup>7</sup> and inserted into an EcoRI-XhoI-cleaved pBR H2A-3 vector lacking the EcoRI-XhoI fragment. The TaqI-XhoI fragments of the resulting recombinants were inserted into the h22 DNA recombinants as described elsewhere<sup>7</sup>.

segment<sup>7</sup> untouched. The distance of the modulator from the TATA box differs in the H2A and the chimaeric H2A<sup>c</sup> gene by only 5 nucleotides and between TATA box and initiator segment by 10 nucleotides, as the exchanged TATA box segments were of nearly identical size (see Fig. 1). The circularized repeat unit, free of vector DNA, was injected into *Xenopus* oocyte nuclei together with  $[\alpha^{-32}P]GTP^{33}$ . Total labelled RNA was isolated and analysed by gel electrophoresis and autoradiography. Expression of the wild-type and mutant H2A genes was monitored in each case against the transcription of the H2B gene which, residing within the same DNA molecule, served as an internal control.

Figure 2b shows that the chimaeric TATA-H2Ac gene produces a novel H2A° mRNA which is resolved into two bands on the electropherogram; the more slowly migrating of these is almost certainly a conformational artefact in this partially denaturing gel system<sup>34</sup> because trimming of the mRNA-DNA hybrids with mung bean nuclease by the modified Berk and Sharp procedure<sup>35,36</sup> produced a single protected DNA band (see Fig. 3b). The TATA-H2Ac gene is transcribed as rapidly as the wild-type H2A gene (Fig. 2a, b). Calculation of the H2A mRNA synthesis by densitometric tracing of the autoradiogram was performed as described elsewhere7. The 5' terminus of the H2Ac mRNA was mapped with mung bean nuclease36 to nucleotide position -66 of the TATA-H2Ac gene (Fig. 3b, e-h) 26 nucleotides downstream of the TATA box (see Fig. 1). In both the wild-type conalbumin and H2A genes, the distance of the initiation site from the TATA box is 28 nucleotides (see Figs 1 and 3, a and b). A hierarchical relationship between the TATA box and the initiator segment<sup>7,8</sup> is again apparent in that the former determines the distance to the initiation site which, however, can be modified by a few nucleotides if a favourable base composition<sup>8</sup> such as CAA or CAT (see Fig. 1) in the sequence ladder is available. The TAGA-H2Ac gene produces what seems to be an identical transcript to the TATA-H2A' mRNA, but at a reduced rate, calculated to be one-fifth of the transcription rate of the TATA-H2A° gene (Fig. 2c). A similar reduction in transcription rate is found for the TATA box deletion mutant (Fig. 2e and ref. 7). The 5' terminus of the TAGA-H2A° transcript has been mapped to the same nucleotide position (Fig. 3d) as the TATA-H2A° mRNA 5' end. This contrasts with the transcripts detected after injection of the TATA box deletion H2A mutant7 which exhibit a pronounced heterogeneity of 5' ends, as seen in Fig. 3c.

Note that the chicken DNA fragment, although differing in sequence from the substituted sea urchin segment except for the TATA box, can nevertheless replace it functionally. In addition, the natural initiation site, although left untouched, is not used for initiation of transcription of the H2A° gene unit, presumably because it is too close to the replaced TATA box. These observations are further evidence for the importance of the TATA box sequence per se, and for its predominance in selecting the first nucleotide(s) of the initial transcripts<sup>7,9,12–14,17</sup>.

This work shows the importance of the TATA box for transcription in vivo, and particularly of the second T in the sequence. A single base change, from T to G, in the third position of the TATA sequence, does not alter the site of RNA chain initiation, but reduces the rate of transcription by a factor of five. A deletion of the TATA box in the H2A gene also decreased transcription fivefold. The in vivo effects of the TAGA box mutation are consistent with the in vitro results, where the T to G mutation decreased the rate of transcription



**Fig. 2** Gel analysis of total RNA from microinjected oocytes. Wild-type and mutant 6-kb DNA circles (4 ng per oocyte) were injected together with 0.3 μCi of  $[\alpha^{-32}P]GTP^{33}$ . The RNA was extracted and analysed as described elsewhere <sup>7,34</sup>. The labelled endogenous *Xenopus* RNAs are visible in the upper sections of the gel slots. a-c, RNA of oocytes injected with h22 wt, TATA-H2A $^c$ , TAGA-H2A $^c$  DNA; d-e, RNA from h22 wt and h22 ΔB (TATA box deletion) <sup>7</sup> DNA. The positions of the histone mRNAs are indicated. The amounts of RNA loaded onto each slot correspond to a sample of two oocytes.

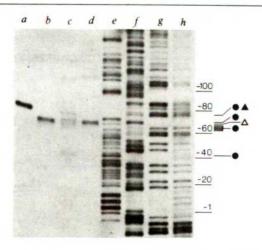


Fig. 3 Mung bean nuclease mapping of the 5' ends of H2A mRNA. For the mapping experiment, total unlabelled RNA from oocytes injected with wild-type and mutant DNAs were purified on CsCl step gradients, hybridized to their cognate 0.9-kb *Hinf-Xho*I DNA fragments<sup>31</sup>, 5'-<sup>32</sup>P-labelled at their *Xho*I site (see Fig. 1) and treated with mung bean nuclease as described elsewhere a-d, Labelled DNA fragments protected by RNA from h22 wildtype, TATA-H2A<sup>c</sup>, h22  $\Delta B$  (TATA box deletion) and TAGA-H2A<sup>c</sup> templates, respectively. The amount of protected DNA fragments in a and b correspond to RNA from 0.3 oocytes; for c and d the amount of DNA fragments loaded onto the slots was increased fivefold to facilitate comparison with a and b. e-h Are the G,A,C,T DNA sequencing reactions<sup>32</sup> of the *Hind-XhoI* DNA fragment of TATA-H2A<sup>c</sup> DNA. The numbering of the nucleotide sequence is that of Fig. 1b. Symbols at the side denote the uncorrected H2A mRNA 5' map positions: ▲, h22 wild-type; △, TATA and TAGA-H2A<sup>c</sup>; and ●, h22 ΔB (TATA box deletion) mRNAs.

20-fold and did not change the site of RNA chain initiation<sup>21,24</sup>. The less drastic reduction of transcription observed with the TAGA box mutant, relative to the in vitro studies, may be due to the activity of the modulator sequences in the in vivo system. Almost all the 5' termini of the TAGA-H2A' transcripts can be mapped to a single (or at least predominant) initiation site with few, if any, transcripts initiating further upstream, while transcripts of TATA-deletion mutants in H2A and other genes quite frequently show a plurality of 5' termini7.9-12. If, as speculated, the TATA box serves to guide the RNA polymerase into a correct initiation frame<sup>7,13</sup>, a T to G mutation may simply promote a less productive 'initiation complex'. Because such a complex may still represent, energetically, the most favoured structure, alternative starting points, readily detected in TATAdeletion mutants, cannot arise.

Some eukaryotic genes known to be expressed at high rate, such as viral 1.4,37 genes and the predominant H4 gene of two sea genes and the predominant H4 gene of two sea urchins5, do not have recognizable or very deformed TATA boxes. It will be of interest to learn what controls initiation of transcription in these cases.

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# Radiation-induced base substitution mutagenesis in single-stranded DNA phage M13

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Radiation-induced mutagenesis of Escherichia coli depends on the ability of the bacteria to perform error-prone repair 1,2. This SOS repair activity is induced by irradiation of bacteria and gives rise to mutations both at the site of radiation-induced lesions (targeted mutations) and in undamaged DNA (untargeted mutations)1,3-6. To elucidate the relative contributions of targeted and untargeted mutations to  $\gamma$  and UV radiation mutagenesis we have determined the DNA sequences of 174 M13 revertant phages isolated from stocks of irradiated or unirradiated amber mutants grown in irradiated (SOS-induced) or unirradiated (non-induced) host bacteria. As reported here, differences in the spectra of base change mutations induced in the various conditions were apparent, but we detected no obvious specificity of mutagenesis. In particular, in our conditions, pyrimidine dimers did not seem to be the principal sites of UV-induced base substitution mutagenesis, suggesting that such mutagenesis occurs at the sites of lesions other than pyrimidine dimers, or is untargeted.

We used single-stranded DNA phage M13 to study independently the effects of target DNA irradiation and the consequences of induction of the SOS repair system. M13 is particularly useful in such an investigation because (1) mutagenesis of single-stranded DNA phages by UV and  $\gamma$ 

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Table 1 Survival and mutagenesis of irradiated and unirradiated M13 grown in UV-irradiated and unirradiated bacteria

	Irradı Phage		No. of expts	Survival	Mutation frequency
CI11	∫ o	0 UV	2	anneau.	$1.6(\pm 0.7) \times 10^{-4}$ $1.3(\pm 0.7) \times 10^{-3}$
am6H1	Įυν	$\overset{0}{\mathbf{U}\mathbf{V}}$	2	3.4(±2.6)×10 <sup>-4</sup> 5.7(±1.0)×10 <sup>-4</sup>	$1.6(\pm 0.3) \times 10^{-4}$ $2.6(\pm 0.9) \times 10^{-3}$
	0	0 UV	5		$7.4(\pm 3.3) \times 10^{-6}$ $3.8(\pm 2.7) \times 10^{-5}$
am7H3	σν	0 UV	2	$1.7(\pm 0.5) \times 10^{-2}$ $2.8(\pm 0.5) \times 10^{-2}$	$7.7(\pm 0.6) \times 10^{-6}$ $1.5(\pm 0.4) \times 10^{-4}$
am7H3	7	$\overset{0}{\mathbf{u}}\mathbf{v}$	5	$4.7(\pm 1.8) \times 10^{-3}$ $7.1(\pm 3.5) \times 10^{-3}$	$3.0(\pm 1.0) \times 10^{-5}$ $3.5(\pm 3.1) \times 10^{-4}$

iog phase cultures of KA798 and KA805 SupD grown with bubbling at 32 °C to an  $A_{560}=0.3$  were divided into two portions, one of which was irradiated with UV light. The cultures were grown in the dark for a further 20 min to maximize SOS induction<sup>4</sup> and were then infected with intact or irradiated M13 phage. After adsorption at room temperature for 3 min the infected bacteria were plated out and incubated overnight at 37 °C. Plaques were counted and revertants picked for DNA sequence analysis. In experiments with am6H1 phage, the UV dose to phage was 90 J m<sup>-2</sup> and to the host bacteria 50 J m<sup>-2</sup>. In experiments with am7H3 phage, phage were irradiated with 60 J m<sup>-2</sup> and the host bacteria with 60 J m<sup>-2</sup>. The  $\gamma$ -ray dose (in broth in air) was 1.5 Mrad. Mutation frequencies are the proportion of revertant phages (titred on KA798) to total phages (titred on KA805 SupD); standard deviations are given in parentheses.

irradiation is dependent on the activity of the same  $recA^+$  and  $lexA^+$  bacterial genes that are required for the induction of all SOS functions in  $E.\ coli$ , including radiation mutagenesis<sup>5.6</sup>, (2) its single-stranded DNA is not subject to error-free repair by excision<sup>7</sup> or by multiplicity reactivation (recombination repair)<sup>8</sup>, and (3) its DNA has been completely sequenced<sup>9</sup> and, as it is single stranded, it is possible unambiguously to identify the exact bases involved in any sequency alteration.

Forty-nine revertants of am6H1 and 125 revertants of am7H3 were isolated from populations of irradiated and unirradiated phages grown in irradiated and unirradiated bacteria (Table 1), and the DNA sequence in the region of the amber codon was determined by the Sanger method<sup>9,10</sup>. Figure 1 shows the number of occurrences of each reversion sequence. Except for the  $TAG \rightarrow GAG$  transversion, which was not found for either amber, all other detectable single-nucleotide changes were observed. ( $TAG \rightarrow TAA$  produces an ochre nonsense codon which would not have been detected as a revertant.) Our failure to detect any GAG revertants might have been due to the infrequency of the  $T \rightarrow G$  transversion or the inability of glutamic acid to substitute for glutamine at these two sites.

The limited number of mutants that can be analysed by DNA sequencing imposes a limit on the significance of differences observed between the spectra of base changes induced by various treatments. An additional problem is that pre-existing mutants may make a significant contribution to the spectra for spontaneous revertants and revertants isolated after those treatments which result in reversion frequencies close to the spontaneous frequency. (Reconstruction experiments showed no significant growth advantage or disadvantage of revertants.) In an attempt to minimize the problems of pre-existing revertants, several stocks of the amber phages were prepared from isolated plaques for each experiment. For those treatments resulting in an elevated reversion frequency, the fact that the revertants were isolated from infective centres ensures that they arose independently, and are therefore more likely to represent accurately the mutational spectrum.

In addition to increasing the frequency of mutations of unirradiated phage, UV irradiation of host cells increased the variety of base changes. Some of the base changes found among these untargeted mutants had not been detected in the spontaneous spectra, while some of the base changes found frequently in the spontaneous spectra were less common in the untargeted spectra. This finding agrees with reports that in addition to increasing the frequency of mutation of unirradiated and irradiated phage, induction of the SOS repair system may alter the specificity of mutagenesis<sup>6,11</sup>. No increase in the mutation frequency was observed when UV-irradiated phage were grown in unirradiated hosts, but a considerable increase was observed when they were grown in UV-irradiated hosts (Table 1)3,4,6. This implies that UV photoproducts in target M13 DNA are not directly mutagenic, but that they are mutagenic when the SOS repair system has been induced. To assess the role of pyrimidine dimers in this mutagenesis we had chosen to study two amber mutants in which the thymine had the potential to be dimerized with a neighbouring thymine in the preceding codon. (Furthermore, in the case of the am6H1 mutant the third base of the preceding valine codon could be replaced by any other nucleotide without changing the sense of this codon.) Few of the UV-induced mutants of am6H1 involved the thymine. On the other hand, the T+C transition seemed to make a major contribution to targeted mutagenesis of am7H3, but this transition also contributed significantly to untargeted mutagenesis of this mutant. The fact that UV-induced mutations frequently involved the adenine and guanine bases of both amber mutants suggests that pyrimidine dimers are not the main sites of base substitution mutagenesis in UV-irradiated M13 DNA. Two alternative possibilities are that photoproducts other than pyrimidine dimers are important targets for UV-induced base substitution mutagenesis or that pyrimidine dimers can have distal mutagenic effects on the same molecule.

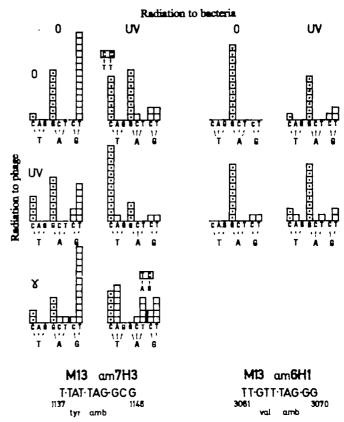


Fig. 1 DNA sequences of revertants of irradiated and unirradiated M13 am7H3 and am6H1 grown in UV-irradiated and unirradiated bacteria. Revertants from the experiments shown in Table 1 were isolated and their DNA sequences in the region of the amber mutation determined by the Sanger method<sup>9,10</sup>. For am6H1 the M13 Hhal fragment 5 (ref. 19) was used as primer, and for am7H3 the M13 Thal fragment 1 (ref. 19) was used After extension by E. coli DNA polymerase I, Klenow subfragment, the primers were removed by Hhal digestion and the products analysed on 12% polyacrylamide gels. Each box corresponds to an independently isolated revertant: D, transition; LXI, transversion; XXI, tandem base changes.

 $\gamma$ -Induced mutagenesis of E. coli depends on the recA<sup>+</sup> and lexA+ gene functions, but seems less dependent on SOSinduced de novo protein synthesis<sup>2</sup>. Consistent with this finding,  $\gamma$  irradiation of am7H3 was mutagenic even when the phage was grown in unirradiated hosts. However, growth of  $\gamma$ -irradiated phage in UV-irradiated hosts increased mutant vields considerably, suggesting that  $\gamma$  irradiation, like UV irradiation, also causes DNA damage whose mutagenic potential is only realized in SOS-induced cells<sup>12</sup>. Insofar as the spectra of base changes induced by y and UV irradiation differ (difficult to assess on the small sample shown here, but see refs 11, 13, 14), these lesions are likely to be different, a predictable conclusion in view of the different DNA lesions caused by UV and y irradiation15

Other investigators (ref. 13 and refs therein) have found that UV light sometimes induces tandem base change mutants whose appearance has been considered as evidence of mutations occurring at the sites of pyrimidine dimers 1.5,13. In our experiments two such mutants were found in phage grown in UVirradiated hosts, but neither of these could be attributed to targeted mutations at the sites of pyrimidine dimers, as one was from unirradiated phage and the other involved two purines. These mutants, and the fact that both UV and  $\gamma$  irradiation induced a wide variety of different base changes in M13 (Fig. 1) and in the lac i gene of E. coli<sup>13,14</sup>, raise the possibility that induction of the SOS repair system, by UV or  $\gamma$  radiation, causes clusters of targeted and untargeted base changes. The observation that non-tandem double base changes in the anticodon sequences of an E. coli tRNA gene occur at much higher frequencies after UV irradiation than expected from the frequency of single base mutants<sup>16</sup> supports this idea.

The contribution of untargeted mutagenesis mutagenesis may be higher for phage genomes than for bacterial genomes, because phage DNA experiences more rounds of replication in the presence of SOS mutator activity<sup>1</sup> and because post-replicative mismatch correction<sup>17</sup> of untargeted mutations may be less efficient for phage DNA. Our data indicate that, in the conditions used, thymine dimers are not the principal sites of UV-induced base substitution mutagenesis of phage M13. If this is a general phenomenon, the photoreversibility of the mutagenic effects of UV on bacteria 18 and the photoreversibility of the UV induction of the SOS repair system<sup>3</sup> may indicate that pyrimidine dimers are primarily inducing lesions for a process (SOS repair) necessary for targeted and leading to untargeted mutagenesis.

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# Preferential cleavage of phage $\lambda$ repressor monomers by recA protease

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Expression of phage lytic functions in  $\lambda$  lysogens of Escherichia coli is prevented by phage repressor, which binds to specific operator sequences on the prophage DNA. Phage induction accompanies the expression of an entire set of 'SOS functions', which occur when host DNA is damaged or DNA synthesis is interrupted. In these conditions,  $\lambda$  repressor is thought to be inactivated by recA protease, thereby leading to expression of phage lytic functions1. We have now characterized the repressor from a  $\lambda$  mutant, ind'-1, which, as prophage, is more easily induced than wild-type phage by a weak inducing treatment2. At high concentrations,  $\lambda ind^3$ -1 repressor differs from wild-type repressor in two ways: whereas wild-type repressor forms dimers and becomes relatively resistant to the recA protease, a much greater proportion of \(\lambda ind^s-1\) repressor remains monomeric and the protein remains sensitive to the protease. These findings support previous indications<sup>3</sup> that repressor monomers are the preferred substrate for protease, both in vivo and in vitro.

Phage  $\lambda$  repressor has two functional and physical domains: an amino-terminal one determining operator recognition and a carboxy-terminal one determining the formation of repressor oligomers<sup>4</sup>. As only dimers of repressor bind to phage operators with high affinity and specificity<sup>4,5</sup>, separation of the two domains by proteolytic cleavage should inactivate the protein, and indeed, the purified N-terminal fragment binds to opertor DNA with 10<sup>4</sup>-fold lower affinity than does intact repressor<sup>4</sup>. In vivo and in vitro the recA protease specifically cleaves repressor into the two domains, thereby inactivating it<sup>1,1</sup>

Two lines of evidence suggest that  $\lambda$  repressor dimers are relatively resistant to the recA protease. In vitro, the fraction of repressor cleaved, as measured by inactivation of repressor DNA binding activity, decreases at repressor concentrations where a significant proportion of repressor exists as dimers<sup>3</sup>. This effect is not due to saturation of the protease, because the rate of cleavage of admixed P22 repressor is unaffected even by high levels of  $\lambda$  repressor<sup>3</sup>. In vivo, induction of  $\lambda$  is deficient in a cell which overproduces repressor, whereas the heteroimmune phage  $\lambda$  imm<sup>434</sup>, present as a prophage, is normally inducible in such cells7

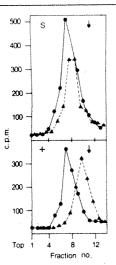
To test further whether repressor dimers are protease resistant, we have characterized the repressor of the hyperinducible mutant,  $\lambda ind^{s}-1$ . Because this mutation lies in the region of cI, which encodes the C-terminus of repressor (see Fig. 1), we thought that \(\lambda ind^s-1\) repressor might be altered in its ability to dimerize and might therefore have increased susceptibility to recA protease at high concentrations.

Two in vivo tests argue against the possibility that  $\lambda ind^{s}-1$ induces more readily because its repressor is defective in operator binding: (1) the mutant can form lysogens as efficiently as λind<sup>+</sup> phage<sup>8</sup>; and (2) while the rate of spontaneous phage release from a  $\lambda ind^{s}-1$  lysogen of a  $recA^{+}$  host is an order of magnitude higher than for  $\lambda ind^+$ , there is no spontaneous phage production by the mutant prophage in a recA host, which lacks recA protease activity (unpublished observations). We conclude that the \(\lambda ind^{\sigma} - 1\) repressor is functionally stable in vivo and likely to be altered in its interaction with the protease.

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1 Sedimentation of \(\lambda ind^\*-1\) S-labelled and \(\lambda\)ind\* repressors. repressor was diluted to  $4 \times 10^{-9}$  M in a buffer containing 0.01 M PIPES pH 6.9, 0.2 M NaCl, 1 mM EDTA, 0.1 mM dithiothreitol, 50 µg ml Jovine serum 10 mg ml<sup>-1</sup> albumin bovine haemoglobin marker (1), either with (A) or without ( unlabelled repressor at 10" Following 90 min incubation at 0 °C to establish the monomer-dimer equilibrium, 0.3 ml of each sample was layered on a 5-ml 5-20% sucrose gradient in the same Gradients were centrifuged in a Beckman SW60Ti rotor for 20 h at 59,000 r.p.m. at 4 °C, 0.3-ml fractions were collected, the position of the haemoglobin marker determined, haemoglobin added to each fraction up to this same peak level to standardize the counting buffer and the radioactivity of each fraction

measured. Bottom panel, ind\*; top panel, ind\*-1. To determine the position of ind\*-1 on the A genetic map, Aind\*-1 sus 029 was crossed with various A  $spi^-$  mutants with deletions which end in cI or left of cI (ref. 11). Because  $spi^-$  phage fail to grow on a  $recA^-$  host,  $sus^+$   $spi^+$  phage produced by recombination between the right end of the spi deletion and sus 0+ could be selected by plaque formation on recA sup bacteria. At least 1,000 of these recombinants were scored for the presence of ind\*-1 by their plaque type on DM1285, on which ind\* and ind\*-1 phage form turbid and clear plaques respectively<sup>12</sup>. These crosses established that ind\*-1 lies between the right ends of spi-23 and spi-91 (ref. 11), placing it in the left one-third of cI or in the right end of rex. This map location was verified by in vitro recombination. To facilitate purification of ind\*-1 repressor, plasmid pKB280 (ref. 13) was modified to overproduce ind\*-1 mutant repressor. A 520-base pair HindIII fragment, which includes the left one-third of the cl gene, was removed from pKB280 to make pBK1; the homologous fragment excised from phage Aind\*-1 was then inserted into pBK1 to produce pBK2. pBK1, which had the portion of cI coding for the NH<sub>2</sub>-terminus of repressor, was resistant to λcI mutants (see also ref. 5). However, pBK1 was sensitive to Avir. Therefore, cells carrying pBK2 could be selected on the basis of their resistance to Avir. The presence of ind\*-1 on pBK2 was confirmed by rescue of ind\*-1 from pBK2 by genetic recombination. Repressor levels in cells carrying pBK2 were lowered by introducing into them F'laciQ1 which overproduces lac repressor<sup>14</sup>. Because the phage repressor genes in pKB280 and pBK2 are fuesed to the lacZ promoter-operator region, the lac repressor reduced the phage repressor levels. After further inducing treatment with mitomycin C, it became possible to superinfect cells with  $\lambda c47$ , which has a clear-plaque mutation near ind\*-1, and thereby to rescue the ind\*-1 alleles by phage plasmid recombination. Phage carrying ind<sup>s</sup>-1 were distinguished from  $\lambda c47$ by production of a turbid plaque on normal indicator C600 and a clear plaque on DM1285, as described previously<sup>12</sup>. Repressor was purified from extracts of cells carrying pKB280 or pBK2 by method (1) of Johnson et al.15 and was estimated to be 98% pure by gel electrophoresis. The mobility of the purified  $ind^*$ -1 repressor in polyacrylamide gels was slightly greater than that of  $ind^+$  (see Fig. 2). By the filter binding assay, the DNA binding activity of one of our preparations of ind\*-1 repressor was found to be twofold less than wild-type repressor purified by the same procedure. While this effect may have resulted from a change in dimer association as described in the text, variability in the same range can be found in different preparations of wild-type repressor 10. Repressor concentrations throughout are given in monomers.

Plasmid pBK2, which overproduces  $ind^s$ -1 repressor, was isolated and used as a source of mutant repressor (see Fig. 1) to be compared with wild-type repressor for its ability to form oligomers and to serve as substrate for recA protease. The repressor concentration in these experiments was in the range  $4 \times 10^{-9}$ - $1.4 \times 10^{-6}$  M, which was achieved by adding varying quantities of unlabelled repressor to a small quantity of <sup>35</sup>S-labelled repressor and incubating for a period of time to allow establishment of equilibrium between monomers and higher oligomeric forms of repressor. Results were analysed by monitoring the labelled repressor. Oligomer formation was compared by measuring sedimentation rates in sucrose gradients.

Wild-type repressor sedimented faster at  $10^{-6}$  and  $10^{-7}$  M than at  $4\times10^{-9}$  M, as expected<sup>9</sup>, indicating oligomerization at the higher concentrations (Fig. 1 and data not shown). By contrast, the sedimentation rate of *ind*<sup>5</sup>-1 repressor did not increase significantly except at the highest concentration

 $(10^{-6} \text{ M})$ . Oligomerization of monomeric  $ind^s$ -1, compared with wild-type, repressor therefore appeared to be far less extensive.

The kinetics of repressor cleavage by the recA protease was then determined in a purified system by measuring one of the cleavage products. Aliquots were taken at various times and analysed on SDS-polyacrylamide gels; repressor concentration varied in twofold steps. The rates of cleavage were estimated by densitometric scanning of autoradiograms (Fig. 2) and are summarized in Table 1. In the experiment shown, excess unlabelled ind+ repressor, added to a low concentration of labelled ind+ repressor, partially protected the latter from cleavage by recA protein. Furthermore, as repressor concentration increased, its rate of cleavage progressively decreased from that expected if it were proportional to repressor concentration. Consistent with previous studies measuring inactivation of repressor and as argued previously<sup>3</sup> (see above), this effect was not due to saturation of the protease, because (1) the same pattern was observed at a higher protease concentration (Table 1) and (2) protease monomers were present in at least a sixfold molar excess over repressor monomers.

By contrast,  $ind^*-1$  repressor was cleaved at a rate nearly proportional to repressor concentration, deviating only slightly at the highest concentration tested  $(1.4 \times 10^{-6} \text{ M})$  (compare Fig. 2a and b; Table 1). As both wild-type and mutant repressors were cleaved at about the same rate  $(4 \times 10^{-9} \text{ M})$ ; Table 1) and both are largely monomers at this concentration (refs 9, 10 and Fig. 1), we conclude that monomers of the two repressors have roughly equal sensitivity to protease. We propose that the  $ind^*-1$  repressor is more sensitive at high concentrations because of its reduced ability to form dimers. These findings therefore provide further evidence that repressor monomers are a preferred substrate for recA protease<sup>3</sup>.

The above properties of  $ind^s$ -1 repressor can probably account for the behaviour of the  $\lambda ind^s$ -1 mutant  $in\ vivo$ . At  $10^{-7}$  M, the concentration estimated to be present in lysogenic cells°, a greater relative amount of  $ind^s$ -1 repressor should be present as monomers, susceptible to the recA protease. Consequently, in a weakly induced cell, in which only a small amount of protease should be activated, a relatively high rate of repressor cleavage would be expected, leading to efficient induction. On the other hand, in a lysogen of wild-type  $\lambda$ , more repressor should be in the form of dimers resistant to cleavage, and should, therefore, bind to operators and maintain the repressed state.

We acknowledge the gift of  $\lambda \ prec A (tif-1) \ c \ I857 \ Q73 \ S7 \ from R.$  Yocum and the communication of unpublished observations

Table 1 Influence of repressor concentration on eleavage rate by the recA

protection					
	Repressor concentration	Rate of repressor cleavage (pmol mi <sup>-1</sup> min <sup>-1</sup> ) of			
$(\mu g m l^{-1})$	(n <b>M</b> )	ind+	ind*-1		
70	4	0.10, 0.15	0.18, 0.20		
	35	0.8, 0.9	1.6, 1.7		
	140	2.0	Selected		
	1,400	11,8	42, 46		
300	35	2.0	measings/		
	140	6	109Addin		
	1,400	24	salaseel		

<sup>35</sup>S-labelled repressor was diluted with buffer or unlabelled repressor, allowed to reach monomer-dimer equilibrium and then incubated with recA protein as described in Fig. 2 legend. At 2.5-min intervals, samples were removed until the reaction had run to completion. The reaction was stopped by adding samples to stopping buffer as described <sup>17</sup> and the samples subjected to polyacrylamide gel electrophoresis. Films exposed to the gels were scanned by a densitometer (Quick Scan R&D, Helena Laboratories) to determine the rate of appearance of label in the upper repressor fragment (R1). The time at which 50% of the maximal level of cleavage had occurred was determined from a plot of film optical density at of cleavage in pmol per ml per min was calculated by multiplying the average fractional rate of repressor cleavage during this time by initial repressor concentration. Values separated by commas are measurements from independent experiments. In the reactions in which recA concentrations were 300 μg ml<sup>-1</sup>, DNA was 25 μg ml<sup>-1</sup>. Varying the ratio of unlabelled to labelled repressor in the reaction mixtures did not influence the results.

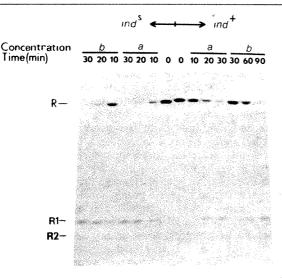


Fig. 2 Influence of repressor concentration on cleavage by recA protein. <sup>35</sup>S-labelled repressor was diluted to  $3.5 \times 10^{-8}$  M in tubes containing 12 mM Tris pH 7.5, 2 mM MgCl<sub>2</sub>, 0.43 mM CaCl<sub>2</sub>, 20 mM NaCl, 43 mM KCl,  $0.54\,\text{mM}$  EDTA,  $1\,\text{mM}$  potassium phosphate pH 7.5,  $2.4\,\text{mM}$  dithiothreitol, 9% glycerol,  $165\,\text{g}\,\text{ml}^{-1}$  bovine serum albumin,  $1\,\text{mM}$   $\gamma$ -thio-ATP and either with (b) or without (a) unlabelled repressor at  $1.4 \times 10^{-6}$  M. ATP and either with (6) or without (2) unlabelled repressor at 1.4 × 10 mer. Following incubation for 90 min at 0 °C to establish monomer-edimer equilibrium, denatured DNA (6  $\mu$ g ml<sup>-1</sup>) was added, the tubes incubated for 5 min at 37 °C, recA (tif mutant) protein added to 70  $\mu$ g ml<sup>-1</sup> and incubation continued at 37 °C. Samples (30  $\mu$ l) were taken at the indicated time and loaded on a 15-25% SDS polyacrylamide gradient gel and electrophoresed. Gels were stained with bromophenol blue, photographed, prepared for fluorography and subjected to autoradiography. R marks the position of the repressor monomer band, R1 and R2 the positions of the carboxyl- and amino-terminal fragments of repressor respectively. ind\*-1 repressor results are shown on the left half of the gel, ind on the right; the centre lanes show uncleaved repressor. Times indicate time of sample removal. Note the later sample times for ind+ repressor cleavage at the high repressor concentration and the increased mobility of  $ind^*$ -1 repressor and its carboxyl-terminal fragment (R1) compared with  $ind^+$  repressor. Repressor bands on stained gels showed intensity changes similar to those on the autoradiogram (not shown). Similar results were also obtained when wild-type recA protein was substituted for the *tif* mutant recA protein. recA protein was purified from extracts of DM1590  $sup^+$  spr-51 lexA3 tif-1 sfiA11 infected with  $\lambda$  precA (tif-1) c1857 Q73 S7 (prepared by R. Yocum and genotype confirmed by us) at a multiplicity of infection of 3. After incubation for 2 h at 37 °C, each litre of cells was collected by centrifugation, suspended in 2 ml 20 mM Tris pH 7.5,  $5 \times 10^{-4}$  M EDTA,  $10^{-3}$  M dithiothreitol, 10% w/v sucrose, 0.2 M NaCl and stored frozen at -20 °C. After thawing, cells were disrupted by sonication and centrifuged for 33 min at 24,000g. recA protein was then purified by polymin P and (NH<sub>4/2</sub> SO<sub>4</sub> precipitation, followed by chromatography on phosphocellulose in potassium phosphate buffer and finally by sedimentation on sucrose gradients as described elsewhere 16. The protein was 99% pure as judged by gel electrophoresis. One A280 unit of recA protein is equivalent to 0.59 mg ml<sup>-1</sup> (J. Roberts, personal communication).

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# recA-independent general genetic recombination of plasmids

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The small circular genomes of several bacteriophages and a number of plasmids have been used extensively to study various aspects of the mechanism of genetic recombination. Several studies have demonstrated that the interconversion between the circular monomers and larger circular oligomers of many plasmid DNAs is mediated, at least in part, by the pathway which carries out genetic recombination in wild-type Escherichia coli<sup>1-4</sup>. The recA, recB and recC mutations block conjugationmediated recombination in E. coli<sup>5,6</sup>. However, Clark and his co-workers7.8 isolated two mutations, sbcA and sbcB, which indirectly suppress the recombination deficiency of recB and recC mutations. These indirect suppressor mutations seem to cause recombination by other pathways which require the recA gene product as well as products of other genes such as recF, recJ and recM, which are not normally required for recombination in E. coli<sup>9,16</sup>. Here we report an investigation of the effect of recombination-deficient mutations on plasmid recombination in E. coli. We show that sbcA mutations induce a new plasmid recombination pathway that is 10-15 times more efficient than the normal wild-type recombination pathway and independent of the recA and recF functions.

The E. coli plasmid pVH51 is a 3.8-kilobase (kb) plasmid which codes for immunity to colicin E1 (ref. 11). It forms small numbers of circular oligomers by recombination in wild-type E. coli strains apparently because it lacks a genetic element required for high rates of recombination<sup>4,12</sup>. To study the effect of various recombination-deficient mutations on the recombination of pVH51 DNA, monomeric pVH51 DNA was purified by electrophoresis on preparative agarose gels and used to transform E. coli AB1157 and a number of isogeneic strains containing different recombination-deficient mutations. Plasmid DNA was then purified from the resulting strains and examined for the presence of oligomeric forms by electrophoresis on agarose gels. The results (Fig. 1a) showed that pVH51 DNA formed very few circular oligomers when maintained in E. coli AB1157 (2-4% by weight, determined by electron microscopy) or other wild-type E. coli strains (results not shown). When pVH51 DNA was purified from recA strains (Fig. 1b), recB recC strains (Fig. 1c), recF strains (Fig. 1d) and recB recC recF strains (Fig. 1e) low numbers of circular oligomers were found (<1% by weight). These mutations reduced the numbers of oligomers but their effect is not pronounced as pVH51 DNA forms few oligomers in wild-type E. coli. When strains containing recB recC sbcA mutations (Fig. 1f), recB recC sbcA recF mutations (Fig. 1g) and recB recC sbcA recA mutations (Fig. 1h) were similarly examined, the pVH51 DNA was found to contain high levels of circular oligomers. These results show that the sbcA mutation stimulated the recombination of pVH51 DNA and that the reaction did not require the recA and recF functions.

Studies on the structure of the oligomeric forms of bacteriophage and plasmid DNAs have identified a novel oligomeric form which looks like a figure-8 (refs 13-17) and has many of the properties of a recombination intermediate postulated by Holliday<sup>18</sup> and others<sup>19</sup> to explain both gene conversion and reciprocal exchanges. Similar DNA molecules have been

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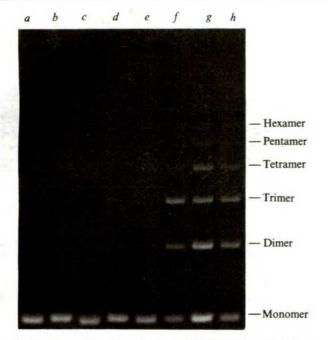


Fig. 1 Electrophoretic analysis of pVH51 DNA purified from isogeneic *E. coli* strains containing recombination-deficient mutations. Monomeric pVH51 DNA was purified from *E. coli* RK1058 recA1 (pVH51) as described elsewhere <sup>37</sup> and the monomeric DNA was further purified by electrophoresis on preparative agarose slab gels<sup>38</sup>. The DNA was then used to transform the *E. coli* strain of interest to resistance to colicin E1<sup>39</sup>. Twelve 10-ml cultures of L-broth were inoculated with a single transformant, grown to a density of 4×10<sup>8</sup> cells ml<sup>-1</sup> at 37 °C and chloramphenicol was added to 250 μg ml<sup>-1</sup>. After shaking for 16 h, plasmid DNA was purified from the cultures and analysed by electrophoresis on a 0.5% agarose slab gel<sup>40</sup>. One representative DNA sample from each strain was further purified by centrifugation to equilibrium in CsCl-Et density gradients<sup>37</sup> and analysed by electrophoresis on a 0.5% agarose slab gel. The DNA samples shown were purified from: a, AB1157 wild type; b, JC2924 recA56; c, JC5519 recB21 recC22; d, JC9239 recF143; e, JC3881 recB21 recC22 recF143; f, JC8679 recB21 recC22 sbcA23; g, JC9610 recB21 recC22 sbcA23.

observed in many systems used to study genetic recombination 20-23. A recombination model for circular genomes which integrates all these observations has been discussed in detail elsewhere24. Electrophoretic analysis cannot easily distinguish between circular oligomers, catenated oligomers and figure-8 molecules. Therefore pVH51 DNA purified from a recB recC sbcA recA strain was examined by electron microscopy. The results (Fig. 2 and Table 1) demonstrated that >95% of the oligomers observed in Fig. 1 were circular oligomers. The level of circular oligomers in recB recC sbcA recA strains was ~20-fold higher than in wild-type strains and 100-fold higher than in recA strains. Low levels of catenated oligomers (Fig. 2e) and figure-8 molecules (Fig. 2f) were also observed. To quantitate accurately the levels of figure-8 molecules present, the pVH51 DNA was converted to a mixture of X-forms (Fig. 2g, h) and linear monomers by digestion with KpnI (one cleavage site

per monomer<sup>25</sup>) in conditions that prevent branch migration<sup>15,24</sup> before mounting the DNA for electron microscopy. The results of this analysis (Table 1) verified the presence of figure-8 molecules in the DNA preparation and suggested that figure-8 recombination intermediates might function in the recombination reaction observed in recB recC sbcA recA strains.

The exact relationship between plasmid recombination and the interconversion of plasmid oligomers is unknown; thus we sought to develop a procedure that would allow a more direct measure of plasmid recombination. Tetracycline-sensitive (tetmutations of the ampicillin-resistant, tetracycline-resistant plasmid pBR322 (ref. 26) were isolated by digesting pBR322 DNA with either BamHI or SalI, filling in the resulting 'sticky ends' with reverse transcriptase, followed by cyclization in blunt end-ligation conditions. The resulting plasmids contained 4base pair (bp) insertions, inactivating the tetracycline-resistance gene and either the BamHI (pRDK2) or SalI (pRDK3) cleavage site. These tet mutations had reversion frequencies of <10 The two plasmid DNAs were then digested to unit-length linear DNA molecules using EcoRI and joined to each other with T4 DNA ligase to construct a circular dimer containing one copy of each of the two different mutant alleles in tandem repeat (pRDK301). When this plasmid DNA is transformed into a recombination-proficient E. coli strain, it should recombine to yield a functional tetracycline-resistance gene at a characteristic frequency and the production of tet' progeny during the growth of the initial tet transformant should be related to the recombination frequency. Control experiments (data not shown) demonstrated that recombination occurred to produce tet progeny and that it resulted in the rearrangement of the inactivated restriction endonuclease cleavage sites with respect to each other. The construction of these plasmids and their use in a fluctuation test27 to determine the frequency of plasmid recombination will be described in detail elsewhere. A similar assay has been used to study SV40 recombination28

To determine the effect of recombination-deficient mutations on plasmid recombination, a set of isogeneic E. coli strains containing different recombination-deficient mutations was transformed to amp with pRDK301 DNA and the production of tet' progeny measured as described in Table 2. The effect of fluctuation27 on the measurements was mitigated by making a number of independent determinations and averaging the results. The results (Table 2) showed that recombination to tet' in wild-type backgrounds (sbcA+) was reduced by recA and recF mutations but was stimulated by recB recC mutations. In contrast, recombination in recB recC sbcA strains was stimulated by recA and recF mutations. The difference in the effect of recA and recF mutations on recombination in wild-type and recB recC sbcA strains was quite striking-recombination in recB recC sbcA recA strains was 11,700 times higher than in recA strains and 500 times higher in recB recC sbcA recF strains than in recF strains. This effect required the sbcA mutation as the recA and recF mutations decreased recombination in recB recC strains. Furthermore, the suppression of recA mutations by sbcA mutations occurred in the presence of functional recB and recC genes.

These results demonstrate that plasmid recombination in wild-type E. coli requires the recA and recF gene products, which is consistent with the findings of others<sup>17,29</sup>. Note that the effect of recF mutations on plasmid recombination differs

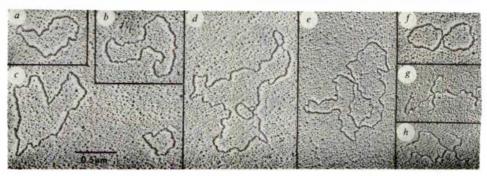
Table 1 Analysis of pVH51 DNA isolated from JC9604 recA56 recB21 recC22 sbcA23

				Distribution	of oligome	ric forms			
	1-mer	2-mer	3-mer	Circular 4-mer	5-mer	6-mer	>6-mer	Catenanes All types	Figure-8s All types
No. (%)* Weight (%)	71.5 48.2	16.1 21.7	7.0 14.1	3.5 9.4	0.4 1.4	0.4 1.7	0.1 0.8	0.7 1.9	0.2 (0.14†) 0.7

<sup>1-</sup>mer, monomer; 2-mer, dimer; 3-mer, trimer, etc.

 <sup>\*</sup> Based on the scoring and measurement of 716 molecules, equivalent to 1,061 monomer units.
 † Based on the scoring of 9,995 linear monomers and 10 X-forms.

Fig. 2 Electron microscopic analysis of pVH51 DNA purified from JC9604 recA56 recB21 recC22 sbcA23. The plasmid DNA preparation analysed in Fig. 1h was treated with y-irradiation to relax the super-twisted DNA molecules or digested with KpnI for 10 min at 10 °C (in conditions supplied by New England Biolabs). After each treatment the DNA was mounted for electron microscopy by the aqueous technique essentially as described elsewhere 13,37. The representative DNA molecules shown are: a, circular



dimer; b, circular trimer; c, circular monomer and circular tetramer; d, circular pentamer; e, catenane of two trimers; f, figure-8 containing two monomer units; and g, h, X-forms produced by KpnI digestion.

significantly from that on conjugation-mediated recombination 9.10. The effect of recB recC mutations is complex as they stimulated genetic recombination of pBR322 but not oligomerization of pVH51. These observations are not inconsistent and are due to the use of two different assays to measure recombination, as we have recently shown that the products of recombination in recB recC strains are almost exclusively circular monomers. These results suggest that the recB recC gene product (exonuclease V) is probably involved in the resolution of recombination intermediates<sup>29</sup>. We have also shown that E. coli strains containing mutations in the gene for topoisomerase I are deficient in plasmid recombination (R.A.F., R.K. and J. C. Wang, unpublished results).

Our results have demonstrated that sbcA mutations induce a recombination pathway that will catalyse plasmid recombination more efficiently than the normal wild-type recombination pathway. Interestingly, this pathway does not require functional recA and recF genes, which suggests that the mechanism of recombination catalysed by it differs considerably from wild-type recombination. Similar results, obtained in studies which examined the effect of sbcA mutations on recombination of bacteriophage  $\lambda$  red, suggest that plasmid and  $\lambda$ recombination may be similar in strains containing sbcA mutations<sup>30</sup>. Presumably, plasmid recombination in recB recC sbcA strains requires proteins coded for by the Rac cryptic prophage as well as other unidentified E. coli gene products31. We have not tested the effect of sbcB mutations on plasmid recombination as these mutations affect the ability of E. coli to maintain plasmids32

Consideration of the data available shows that the recombination-deficient mutations have different effects depending on the type of E. coli recombination event analysed 6,9,10,17,29, These observations suggest that the different homologydependent recombination events differ structurally and there-

Table 2 Effect of recombination-deficient mutations on recombination of

		Ger	notype tested		
				Proportion of	tet <sup>r</sup> progeny
recA	recB	recC	recF	$sbcA^+$	sbcA23
+	+	+	+	$6.4 \times 10^{-4}$	ND
56	+	+	+	$8.1 \times 10^{-7}$	$5.5 \times 10^{-4}$
56 +	+	+	143	$1.6 \times 10^{-5}$	ND
+	21	22	+	$2.9 \times 10^{-3}$	$3.1 \times 10^{-3}$
56	21	22	+	$2.1 \times 10^{-5}$	$9.5 \times 10^{-3}$
+	21	22	143	$4.3 \times 10^{-4}$	$7.9 \times 10^{-3}$

The *E. coli* strain of interest was transformed to ampicillin resistance with pRDK301 DNA<sup>39</sup>. A 10-ml culture of L-broth containing 50  $\mu g$  ml<sup>-1</sup> ampicillin was inoculated with an entire transformant colony and incubated at  $37^{\circ}$ C with shaking until a cell density of  $5 \times 10^{7}$  was obtained. Samples were then plated out on L-broth plates containing either  $50 \, \mu \mathrm{g} \, \mathrm{ml}^{-1}$  ampicillin or  $50 \, \mu \mathrm{g} \, \mathrm{ml}^{-1}$  ampicillin +25  $\mu \mathrm{g} \, \mathrm{ml}^{-1}$  tetracycline to determine the proportion of Tet' cells present in the culture. The values shown are the average results obtained from an analysis of 10 independent cultures. The strains tested are those described in the Fig. 1 legend, and RK1064 recA56 recB21 recC22 and RK1289 recA56, sbcA23 All are isogeneic with E. coli AB1157. ND, not determined.

fore require different proteins to catalyse them. It also seems likely that proteins like exonuclease V (the product of the recB and recC gene 35,36) may function differently in different recombination events. This could explain why recB recC mutations affect the formation of stable recombinants in topologically similar  $\lambda \times F'$  and plasmid crosses differently<sup>33</sup>. The nature of the significant differences between all these recombination events is not fully understood.

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## In vivo decoding rules in Schizosaccharomyces pombe are at variance with in vitro data

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On the basis of in vitro experiments, conflicting hypotheses have been proposed for the assignment of tRNA isoacceptors to the reading of synonymous codons<sup>1,2</sup>. Here we present a genetic approach for studying the decoding capacity of serine isoacceptors in vivo. Cells of Schizosaccharomyces pombe have been depleted of tRNA Ser by mutating the two genes known to code for this isoacceptor, sup3 and sup9, first to UGA nonsense suppressor alleles and then to suppressor inactive alleles carrying secondary lesions. The lethality resulting from the combination of inactive alleles at both loci indicates that tRNA Ser local is essential for growth. Disregarding the remote possibility that this tRNA has vital roles other than in protein synthesis, we conclude that its function in translating UCA cannot be taken over by any other serine tRNA, including the major inosinecontaining isoacceptor. This is at variance with both the wobble rule for inosine proposed by Crick<sup>1</sup> and the 'two out of three' reading scheme proposed by Lagerkvist<sup>2</sup>, and suggests that decoding rules deduced from in vitro data do not necessarily apply to the in vivo situation.

The wobble hypothesis of Crick states that some bases at the first anticodon position can pair with the third codon base in a non-standard way, allowing UG, GU, IU, IC and IA pairs beside the standard ones<sup>1</sup>. Experimental support for the wobble rules is provided by triplet binding assays with *Escherichia coli* ribosomes<sup>3</sup>. However, exceptions to this rule have been described: extended wobbling is observed in mitochondria where U recognizes codons ending with U, C, A and G (refs 4-6). The same seems to be true for a tRNA<sup>Gly</sup> in *Mycoplasma mycoides*<sup>7</sup>. On the other hand, some modifications of U in the wobble position have been proposed<sup>8</sup> to restrict the recognition to codons ending with A.

A further discrepancy (the subject of the present study) concerns the wobble rule for inosine. In the eukaryotes studied so far a major tRNA<sup>Ser</sup> with the anticodon IGA was found and thought to be responsible for the recognition of the codons UCU, UCC and UCA<sup>9,10</sup>. However, in Saccharomyces cerevisiae<sup>11</sup> and Schizosaccharomyces pombe<sup>12</sup> a UCA-reading tRNA<sup>Ser</sup> with the anticodon U\*GA has also been identified. We present here genetic evidence which suggests that in S. pombe, this minor isoacceptor evolved because the major serine tRNA is unable to translate UCA in vivo.

Our approach was to deplete cells of a specific active tRNA by genetic means and to observe the consequences on cell growth. The genes  $sup3^+$  and  $sup9^+$  in wild-type strains of S. pombe code for the tRNA<sup>Ser</sup> isoacceptor having the anticodon U\*GA. This is inferred from genetic evidence<sup>12</sup> and from the nucleotide sequence of the UGA-reading sup3-e opal suppressor tRNA<sup>ser</sup> (e for efficient; anticodon U\*CA, where U\* is partly 2-thiouridine and partly 5-(methoxycarbonylmethyl)-uridine)<sup>13</sup>. A corresponding situation holds for the tRNA Leu isoacceptor (anticodon U\*AA) which is coded for by the genes sup8+ and sup10<sup>+</sup>. Mutations at the anticodon site of these two genes lead to efficient opal suppressor alleles and the product of one of these (sup8-e tRNA) has been sequenced<sup>14</sup>. Strains carrying such opal suppressors thus produce either a tRNA ser or a tRNA<sup>Leu</sup> whose anticodon U\*CA no longer recognizes UCA or UUA, respectively. All four opal suppressors have been inactivated by secondary mutations that map within the respective suppressor genes at different sites 15. These secondary lesions are likely to abolish either the biosynthesis or function of the suppressor tRNAs.

By crossing strains carrying inactive suppressor alleles at, different loci, recombinants harbouring the mutant alleles at both loci can be obtained (Table 1). Three types of tetrads are observed in such crosses and they occur in frequencies expected for a two-factor cross of randomly assorting genes: 1/6 parental ditype tetrads (PD), 2/3 tetratype tetrads (T) and 1/6 nonparental ditype tetrads (NPD). The four suppressor genes studied are not linked genetically to each other. The PDs consist of four colonies. In tetratypes and NPDs, respectively, one and two recombinant spores that carry two inactivated suppressor genes coding for the same tRNA (that is, sup3-e, rX sup9-e, rX or sup8-e, rX sup10-e, rX; r for reversion) fail to form colonies on yeast extract agar. In fact they are unable to undergo the first cell division. Whereas tRNA<sup>ser</sup>-deficient spores elongate to form structures resembling a premitotic stage,  $tRNA^{\mathrm{Leu}}$ -deficient spores do not germinate at all. Identical results were obtained when the spores were placed on minimal agar supplemented with adenine (the only requirement of the strains involved) and incubated at 25°C as well as at standard 30°C (data not shown). Even when one or both partners of a cross carry an active suppressor gene (no inactivating second-site mutation present in one or both suppressor genes of a given pair), recombinants lacking wild-type alleles fail to grow. However, strains carrying two active suppressors inserting different amino acids (for example, sup3-e sup8-e) are viable 16. Thus the lethality observed in combinations of two mutated tRNA<sup>Ser</sup> genes and two mutated tRNA Leu genes, respectively, is due to the loss of the corresponding wild-type tRNAs and not due to excessive suppression.

The indispensable nature of tRNA <sup>Leu</sup><sub>UCA</sub> and tRNA <sup>Leu</sup><sub>UUA</sub> demonstrated by these results is interpreted to mean that in S. pombe, these are the only tRNAs able to read UCA and UUA, respectively with an efficiency sufficient for cell growth. The alternative explanation which assumes an essential function of the specific tRNA <sup>Leu</sup><sub>UCA</sub> and tRNA <sup>Leu</sup><sub>UUA</sub> isoacceptors in processes unrelated to protein synthesis cannot be excluded at present, but seems unlikely.

As our data indicate that in S. pombe the codon UCA is read efficiently only by the tRNA<sup>Ser</sup> with the anticodon U\*GA (a minor isoacceptor), we have purified the major tRNA<sup>Ser</sup> from this organism. We are now sequencing this tRNA and HPLC of tRNA digests<sup>17</sup> has revealed that it indeed contains inosine (our unpublished results). The lack of efficient UCA recognition by the inosine-carrying S. pombe serine tRNA is paralleled by the observation made in S. cerevisiae that no serine-inserting ochre suppressor tRNAs were found to be derived from the inosine-carrying isoacceptor<sup>11</sup>. We conclude from this that in both yeasts, wobbling of inosine when present in the first position of the anticodon IGA of the major tRNA<sup>Ser</sup> isoacceptor permits recognition of the serine UCX codons ending with U or C only.

The finding that the codon UCA is efficiently translated only by the serine tRNA containing the anticodon U\*GA also argues against decoding of the serine codon family UCX by the 'two out of three' method proposed by Lagerkvist<sup>2</sup> for those families of codons that differ only in the third position and code for a single amino acid. In this hypothesis, which is based on *in vitro* protein synthesis experiments, tRNAs pairing in a standard way at the first two codon positions are predicted to read all four codons of the family, irrespective of the nature of the wobble pair.

Similar data excluding decoding by the two out of three method have been reported for the UCG codon in S. cerevisiae, where it was demonstrated that tRNA<sup>Ser</sup> with the anticodon CGA is essential<sup>18,19</sup>. The same situation probably holds for S. pombe, as suggested by preliminary results obtained with a UGA suppressor derived from the gene coding for this tRNA (H. Amstutz, personal communication). In both yeasts the gene coding for the minor tRNA<sup>Ser</sup><sub>UCG</sub> has been sequenced<sup>11,20</sup>. Suppressors derived from it are haplo-lethal in both organisms. The essential nature of the tRNA<sup>Ser</sup> with the anticodon CGA which is demonstrated by this finding also shows that the hypermodified uridine at the wobble position of tRNA<sup>Ser</sup><sub>UCA</sub> prevents reading of the codon UCG. This agrees with earlier in

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Table 1 Analysis of pairwise crosses between S. pombe strains with inactivated serine tRNA and leucine tRNA genes

			Tetrad types			
	Cross	4 colonies (PD)	3 colonies and 1 lethal spore (T)	2 colonies and 2 lethal spores (NPD)	Tetrads with superimposed growth defects	Total tetrads analysed
tRNA <sup>Ser</sup>				•		,
sup3-e,r8×	sup9-e, r49	3	10	1	2	16
r15×	r101	3	10	1	2	16
<i>r30</i> ×	r104	1	9	2	4	16
<i>r36</i> ×	r49	1	12	2	1	16
r40×	r68	2	10	3	1	16
To	otal	10	51	9	10	80
tRNA <sup>Leu</sup>						
sup8-e, r54 ×	sup10-e, r7	2	10	2	0	14
r54×	r17	2	11	0	1	14
r54×	r24	3	7	4	0	14
r61×	r9	5	8	0	1	14
r139×	r15	0	10	3	1	14
r169×	r17	1	10	3	0	14
r440×	r24	1	6	2	5	14
r463×	r34	3	9	0	2	14
To	otal	17	71	14	10	112

The genetic methods used are described elsewhere 15. All strains involved carried the opal nonsense mutation ade6-704 which causes an adenine requirement and the accumulation of a dark red pigment in cells grown on media containing limiting adenine concentrations (for example, yeast extract agar on which asci were dissected). Active opal suppressors (sup3-e, sup9-e, sup9-e, sup9-e and sup10-e) combined with ade6-704 give prototrophic and white colonies, but the secondarily inactivated suppressors (supX-e, rY) have no effect on the ade6-704 phenotype except in the following cases: sup3-e, r8, -r36, sup8-e, r61, -r440, -r463, sup10-e, r17 and -r24. These strains display a pink rather than dark red pigment but are nevertheless auxotrophic. The segregations of colour differences in such cases are in complete agreement with the interpretation that the lethal spores contain mutant suppressor alleles at both loci involved. A minority of tetrads displayed in addition to the spore lethality caused by tRNA depletion, a superimposed lethality of unknown nature. The two types could be distinguished by microscopic examination, as non-growers due to tRNA defects had a constant, characteristic morphology

vitro data8 which indicate that certain modifications of U in the wobble position restrict a tRNA to the recognition of codons ending in A

Further evidence against the hypothesis of two out of three reading has recently been presented by Murgola and Pagel<sup>21</sup>, who showed that in E. coli the glycine codon GGA is read in vivo only by  $tRNA^{Gly}$  with the anticodon NCC, where N is a partially modified uridine<sup>22</sup>. The finding that in S. pombe the codon UUA is read efficiently only by the tRNA Leu with the anticodon U\*AA contrasts with earlier in vitro protein synthesis data obtained for yeast and E. coli tRNA which suggested that UUA may be read by more than one leucine isoacceptor<sup>23,24</sup>

Clearly, in vivo decoding must be studied in more detail, but available evidence suggests that it does not simply conform to the hypotheses proposed on the basis of in vitro data.

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## Wild-type tRNA<sub>G</sub><sup>Tyr</sup> reads the TMV RNA stop codon, but Q base-modified tRNA<sub>0</sub><sup>Tyr</sup> does not

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Although protein synthesis usually terminates when a stop codon is reached along the messenger RNA sequence, there are examples, mainly in viruses, of the stop codon being suppressed by a tRNA species. A strong candidate for this phenomenon occurs in tobacco mosaic virus (TMV) in the form of two proteins (110K and 160K, of molecular weights 110,000 and 160,000, respectively)<sup>1</sup>, sharing an N-terminus sequence, which are translated in vitro from a purified species of viral RNA. We have investigated the identity of the tRNA responsible for production of the 160K protein and show here that it is one of the tyrosine tRNAs. Another tyrosine tRNA, in which the first base of the anticodon is highly modified, does not act as a suppressor, indicating the possible regulatory function of such modifications.

As an assay system for readthrough, we have used simultaneous injection of TMV RNA and amber or ochre suppressor tRNAs from yeast into Xenopus oocytes; this results in synthesis of the 110K and 160K proteins<sup>2</sup>. In the process of screening RPC-5 fractionated Drosophila melanogaster tRNAs (21 suppressor stocks and Oregon wild type) for putative amber or ochre suppressors, we found that one fraction  $(c_1)$  contained a strong readthrough activity in every RPC-5 profile tested, including the wild type (Fig. 1a, b). The fact that this readthrough activity is contained in every tRNA preparation indicated that we had discovered a natural suppressor tRNA for the TMV RNA 110K protein stop codon. Aminoacylation shows that this RPC-5 fraction contains a tRNA Tyr isoacceptor (Fig. 1a), a good candidate for such a suppressor. Purification of this tRNA to homogeneity<sup>3</sup> and subsequent injection with TMV

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RNA unambiguously demonstrates that the synthesis of the 160K protein is stimulated proportionally as a function of the concentration of this tRNA in the oocyte (Figs 2, 3). Injection of tRNATyr isolated from yeast4 produces the same results (Figs 2, 3). This finding can also be reproduced in vitro by addition of tRNATyr to a TMV RNA-primed reticulocyte extract5. Hence tRNA Tyr acts as a natural suppressor tRNA in the production of TMV proteins and is probably responsible for the synthesis of the 160 K protein in tobacco plants as well as in in vitro and in vivo TMV RNA translation experiments where this protein has always been observed 1,6. This interpretation is supported by the fact that the readthrough protein is synthesized in different protein synthesizing systems and with different tRNA components, such as the Xenopus oocyte<sup>2,6</sup>, wheat germ and reticulocyte extracts, and TMV-infected tobacco plants, A minor tRNATyr eluting at higher NaCl concentrations from the RPC-5 column (Fig. 1) can also induce the synthesis of the readthrough protein<sup>5</sup>, but none of the following purified tRNAs could produce this effect: tRNA<sub>G</sub><sup>Asp</sup>, tRNA<sub>G</sub><sup>Asn</sup>, tRNA<sub>G</sub><sup>Glu</sup>, tRNA<sub>G</sub><sup>His</sup>, tRNA<sub>G</sub><sup>Phe</sup> and tRNA<sub>4</sub><sup>Val</sup>, all from *Drosophila*, and tRNA<sub>S</sub><sup>Ser</sup> from yeast<sup>5</sup>. Furthermore, the *Xenopus* oocyte is not sensitive to artefacts2, such as enhanced synthesis of the 160K protein by addition of spermidine or increasing the Mg<sup>2</sup> concentration10

Amber and ochre suppressor tRNA<sup>Tyr</sup> preparations contain about 1/8th suppressor tRNA<sup>Tyr</sup> and 7/8ths wild-type tRNA<sup>Tyr</sup>, reflecting the fact that only one of the eight tRNA<sup>Tyr</sup> genes present in yeast has mutated to a suppressor, and that these two kinds of tRNA cannot be separated from each other<sup>11</sup>. Hence, the readthrough induced by tRNA<sup>Tyr</sup>-derived amber or ochre suppressors is due to the superimposed effect of the suppressor tRNA<sup>Tyr</sup> and the wild-type tRNA<sup>Tyr</sup> (Fig. 3). Unambiguous and independent proof that the TMV RNA stop codon can be read by amber and ochre suppressor is provided by the finding that amber and ochre suppressor tRNA<sup>Ser</sup> induce readthrough, whereas wild-type tRNA<sup>Ser</sup> does not<sup>2,10</sup>.

TMV RNA has only been partially sequenced<sup>12</sup>. Unfortunately, the sequence in the region of the 110K protein stop codon is not known. However, there are strong indications for the presence of an amber (UAG) stop codon. Amber and ochre suppressor  $tRNA^{Tyr}$  and  $tRNA^{Ser}$  can induce readthrough in  $vitro^{2,10}$  and in the *Xenopus* oocyte<sup>2</sup>; amber tRNA shows a slightly enhanced readthrough activity compared with ochre  $tRNA^1$  (see also Fig. 3); yeast  $tRNA^{Tyr}$  can misread amber codons in a synthetic mRNA and in a  $Q_{\beta}$  coat protein amber nonsense mutant, but not in a  $Q_{\beta}$  ochre coat protein nonsense mutant<sup>13</sup>. Thus, the first two letters of the codon must be UA, and UGA is excluded.

Two other interpretations might be considered: pausing <sup>14</sup>, involving a tyrosine codon as a pseudo stop codon, and out of phase reading <sup>15</sup>. However, pausing seems improbable because termination occurs 10–20 times more frequently at this site than does elongation. Furthermore, it is not obvious why yeast amber and ochre suppressor tRNA <sup>Ser</sup> should enhance the readthrough effect<sup>2,10</sup>, as unconventional base pairing would be required in this case. Out of phase reading also seems improbable because it would lead only to a tyrosine codon (UAU) if the frame is shifted by –2 and if a XUA (Leu, Ile, Ser) codon preceded the stop codon (UXX). It also seems unlikely that the out of phase reading product and the readthrough induced by suppressors would have the same length, which is indeed what is observed<sup>2</sup>.

If an amber codon exists, translation by a wild-type tRNA<sup>Tyr</sup> necessitates a G-G pairing at the third position of the codon, a pairing which is forbidden by the wobble rules<sup>16</sup>. Thus, our results suggest that only the first two bases are relevant for reading through the TMV RNA stop codon, similar to the 'two out of three' reading proposed by Lagerkvist<sup>17</sup>. However, Lagerkvist's hypothesis excludes codons of the UA family from this kind of ambiguous reading.

Drosophila melanogaster contains two major tRNA<sup>Tyr</sup> isoacceptors<sup>18</sup> (Fig. 1a) which are probably homogeneic and only differ in the presence or absence of the highly modified base Q in the first position of the anticodon<sup>19</sup>. The full sequence of these isoacceptors has not yet been determined, although preliminary sequencing data confirm this assumption (W. Brunner, M. Altwegg and E. K., unpublished). The  $tRNA_Q^{Vr}$ -containing RPC-5 fraction ( $b_2$ ) does not stimulate the synthesis of the 160K protein (Figs 1-3). Only very high concentrations of  $tRNA_Q^{Vr}$  are able to increase the level of the 160K protein slightly above background (Fig. 3). The presence or absence of the Q base modification does not affect the rate or extent of protein synthesis in  $vitro^{20}$ . The  $tRNA_Q^{Vr}$  used in this experiment has not been inactivated (for example, by a nick in the anticodon) because only a single band in the tRNA region can be observed on a 7 M urea polyacrylamide gel<sup>3</sup>. Thus, degree of  $tRNA_Q^{Tvr}$  modification in the first position of the anticodon regulates the synthesis of the 160K protein.

The fact that eukaryotic tyrosine tRNA is the only one out of 170 sequenced tRNAs that possesses a modified nucleotide in the second letter of the anticodon<sup>21</sup>, a favourable codon context<sup>22-26</sup> and the secondary structure of the TMV RNA

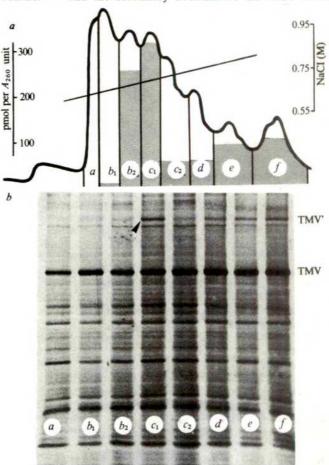


Fig. 1 Correlation of tyrosine acceptor and readthrough activities of RPC-5 fractions. A, Absorption profile (A<sub>260</sub>) of a RPC-5 chromatography<sup>31</sup> (gradient 0.55–0.90 M NaCl) of 200 A<sub>260</sub> units of crude wild-type Drosophila tRNA. The tRNA<sup>Tyr</sup> content of fractions a-f was determined by aminoacylation with <sup>14</sup>C-tyrosine<sup>19</sup> (shaded area, given in pmol per A<sub>260</sub> unit). The two major tRNA<sup>Tyr</sup> isoacceptors are localized in fraction b<sub>2</sub> (tRNA<sub>O</sub><sup>Tyr</sup>) and c<sub>1</sub> (tRNA<sub>O</sub><sup>Tyr</sup>), respectively. A minor tRNA<sup>Tyr</sup> isoacceptor is found in fractions e and f. B, Autoradiography of a 7.5% SDS-polyacrylamide gel of <sup>35</sup>S-methionine-labelled Xenopus laevis oocyte proteins after injection of TMV RNA and RPC-5 fractions a-f. The probes were precipitated with ethanol after RPC-5 chromatography, dissolved in 0.1 M Na acetate and reprecipitated with ethanol to remove the salt. The tRNA concentration injected per oocyte was 0.6–1.2 μg, the TMV RNA concentration 100 ng. At least seven oocytes were injected for each tRNA fraction. Labelling with <sup>35</sup>S-methionine, extraction of the proteins and SDS-polyacrylamide gel electrophoresis was done as described earlier<sup>2</sup>. TMV = 110K TMV protein; TMV = 160K TMV readthrough protein. Readthrough activity above background level is found in fraction ε<sub>1</sub> containing tRNA<sub>O</sub><sup>Tyr</sup> (anticodon GΨA). Fraction b<sub>2</sub> containing the Q base-modified tRNA<sub>O</sub><sup>Tyr</sup> (anticodon GΨA) isoacceptor does not induce readthrough above background level. A slight increase of readthrough activity is associated with the minor tRNA<sup>Tyr</sup> isoacceptor in fractions e and f.

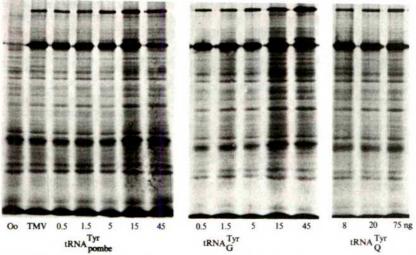


Fig. 2 Autoradiography of 7.5% 5D5-polyacy of 35M-methionine-labelled *Xenopus* oocyte proteins after tRNATyr isolated from Schizosaccharomyces pombe and D. melanogaster respectively. Experimental procedures are as described in Fig. 1 legend. Synthesis of the 160K TMV readthrough protein is stimulated proportionally as a function of the concentration per oocyte of  $tRNA_{pombe}^{Tyr}$  or  $tRNA_{qombe}^{Tyr}$  (anticodons  $G\Psi A$ ) isolated from  $Drosophila^3$ , respectively, whereas  $tRNA_{qombe}^{Tyr}$  (anticodon  $Q\Psi A$ ) isolated from  $Drosophila^3$  is unable to induce this readthrough event. All three tRNAs were at least 95% pure3.

might contribute to production of appreciable amounts of the 160K protein. These circumstances might also be responsible for the approximately equal efficiency in the reading of the TMV RNA stop codon by wild-type tRNA<sup>Tyr</sup> and amber or ochre suppressor tRNA<sup>Tyr</sup> (Fig. 3 and ref. 2). From the above we conclude that the leakiness of the TMV RNA stop codon is due to the misreading of an amber stop codon by a wild-type tRNATyr. This is analogous to readthrough of the UGA termination codon of the coat protein of phage QB, thus producing a product indispensable for infection in Escherichia coli<sup>27-29</sup>

Geller and Rich30 have proposed that the readthrough phenomenon might represent a control of gene expression at the level of termination. They pose the question of whether readthrough of certain proteins occurs, for example, in certain developmental stages of an organism, or after transformation of cells. In fact, significant changes can be observed in the extent of Q base modification in tRNAs isolated from different ontogenetic stages in D. melanogaster 19,31. In transformed mammalian cells the Q base modification disappears almost completely<sup>32</sup>. Whether these changes reflect any activities, as suggested by Geller and Rich<sup>30</sup>, is not known. Putative changes in the extent of Q base modification of the tRNAs in TMVinfected tobacco plants have yet to be demonstrated. Thus, gene expression could be regulated by a single modification enzyme.

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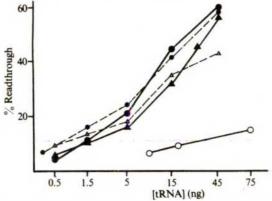


Fig. 3 Dependence of the amount of the 160K TMV readthrough protein on the concentration of various tRNAs. Experimental procedures are as described in Fig. 1 legend and by M.B. et al.<sup>2</sup>. ♠, tRNA<sup>Tyr</sup> (anticodon GΨA) from Drosophila<sup>3</sup>; ♠, tRNA<sup>Tyr</sup> (anticodon GΨA) from S. pombe<sup>4</sup>; •, Saccharomyces cerevisiae amber suppressor tRNA<sup>Tyr</sup> (ref. 2); △, S. cerevisiae ochre suppressor tRNA<sup>Tyr</sup> (ref. 2); ○, tRNA<sup>Tyr</sup>.

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## E. coli ribosomal protein L10 inhibits translation of L10 and L7/L12 mRNAs by acting at a single site

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The genes coding for ribosomal proteins L10 and L7/L12 and for the subunits for RNA polymerase  $\beta$  and  $\beta'$  are clustered within the same transcription unit ( $\beta$  operon) located at the 88-min region of the Escherichia coli genome 1,2. The regulation of these genes is of particular interest because of the ratio of synthesis of the proteins (copy ratio L10:L7/L12: $\beta$ : $\beta'$ = 1:≥4: 0.2: 0.2) and because synthesis of the ribosomal proteins and of  $\beta$  and  $\beta'$  is apparently subject to different regulatory signals (see ref. 3 for a review). It was previously shown that certain ribosomal proteins, including L10, can inhibit the translation of their own mRNA and of mRNA of other genes from the same transcription unit<sup>4-11</sup>. This regulation is physiologically significant in coordinating the synthesis of ribosomal proteins and ribosomes. Although L10 was previously shown to inhibit its own translation in vitro5, the mechanisms for regulation of L7/L12 synthesis have remained unknown. We report here the results of in vivo and in vitro investigations demonstrating that L10 regulates L7/L12 synthesis, as well as its own synthesis, by acting near the L10 translational start site.

1 08

0.94

0 99

We examined the feedback regulatory properties of L10 in vitro using a DNA-directed protein synthesizing system with various DNA templates containing all or portions of the  $\beta$ operon (Fig. 1). When a template containing the entire  $\beta$  operon  $(\lambda rif^{d}18)$  was used, addition of purified L10 to in vitro reaction mixtures specifically inhibited synthesis of L10 and L7/L12 but did not inhibit synthesis of  $\beta$  and  $\beta'$  or of any other protein (Fig. 2, lanes 1-4; Table 1). By transcribing λrif<sup>d</sup>18 DNA, extracting the mRNA and then using the mRNA to direct protein synthesis, we found, as have others<sup>5</sup>, that the inhibition by L10 occurs at the level of mRNA translation (data not shown). When a HindIII DNA fragment containing only the distal portion of the L10 gene, the entire L7/L12 gene and a proximal portion of the  $\beta$  gene was used to direct protein synthesis, addition of L10 did not affect the synthesis of L7/L12 (Fig. 2, lanes 5, 6). We therefore conclude that L10 acts at a single site and inhibits both L10 and L7/L12 synthesis and that this site is in the promoterproximal portion of the  $\beta$ -operon transcript.

When a template (pN01525) containing the entire L10 structural gene and only a portion of the L10 leader sequence was used to direct L10 synthesis, addition of L10 caused marked inhibition of L10 synthesis (Table 1). This result indicates that the 5'-proximal 186 nucleotides of the mRNA are not essential for the repressor action of L10. Using two additional DNA templates, it was also demonstrated that the repressor action of L10 does not require the presence of the distal portion of the L10 cistron. The template  $\lambda\beta$ -trp-lac1, made up of DNA of a hybrid 'trp-lac'  $\lambda$  phage carrying the  $\beta$ -operon promoter and the proximal portion of the L10 gene fused in phase to the distal portion of the trpB gene, directed synthesis of an L10-trpB fusion protein; the synthesis was specifically inhibited by L10 (Table 1). Similarly, specific inhibitory action of L10 was demonstrated using an in vitro system in which the synthesis of an L10-β-lactamase fusion protein was directed by a plasmid DNA template, pFM20, that includes the promoter and only the proximal portion of the L10 gene fused in phase to the  $\beta$ lactamase gene. These results, summarized in Table 1, imply that the target site for L10 repressor action is located between a portion of the L10 leader sequences and the proximal portion of the L10 gene, as shown in Fig. 1. This region includes a possible stem—loop structure in the leader mRNA<sup>12</sup> containing four point mutations<sup>13</sup> that prevent L10 synthesis and which were thought to be involved in the feedback regulatory mechanism for regulation of L10 synthesis. However, two such mutants are in a portion of the leader sequence (at positions 1,515 and 1,516) not required for feedback inhibition. Using a similar in vitro system, Brot et al.<sup>5</sup> found that L10 inhibited translation of L10

Fig. 1 Genes carried by  $\lambda nf^{*1}18$  and construction of plasmids used in this study. The hatched regions of  $\lambda nf^{*1}18$  represent  $\lambda$  DNA and the open regions bacterial DNA. Some of the bacterial transcription units are indicated above  $\lambda nf^{*1}18$  as a P (promoter) and a horizontal arrow indicating the direction of transcription. Transcription of the  $\beta$  operon mitiates approximately at position 1,348 (±1) (ref. 12) Below  $\lambda nf^{*1}18$  is an expanded portion of the transducing phase that denotes the relevant bacterial region. Various restriction enzyme sites are indicated below the expanded region. Only the restriction enzyme sites relevant to the present study are indicated. The complete DNA sequence of this region has been determined and can be found in refs 12, 22. The numbers below the vertical arrows

inslation of L10		λ	rlf <sup>d</sup> 18	
	Pa PLII	PrrnB "		
/// B' BL7	NTIS MO	Y////	7//////////////////////////////////////	////
	[17/12]	+P <sub>β</sub>	PLII (	
1 3524	†† † † 2,444 2,415 2,154 1,792	† †	280	† EcoRI † Pari † HoseIII
Pour	POR		Part of the second	† Hins III
pN02020	(pNO1525)	pF <b>M</b> 20	pGBABR2	

representing restriction enzyme sites indicate the nucleotide position of the restriction enzyme cut using the numbering system of ref. 12. Various portions of λrh\*18 were used to construct hybrid plasmids (shown in the figure) that carry regions of the L11 and β operons. Some of the restriction sites are shown on the plasmids so that the cloned region can be compared with λrh\*18. Plasmid pNO2020 is a derivative of pVH51 (ref. 23) and contains two copies of an EcoRI fragment (nucleotides 2,444-3,524) from λrh\*18 and one copy of another EcoRI fragment from λrh\*18 (nucleotides 2,444-3,524). For m stro studies, the Hhedilli fragment that contains the L7/L12 gene was purified from pNO2020 and used to direct protein synthesis. Plasmid pRZ4006 was used as the parental plasmid for construction of pNO1525. The plasmid pRZ4006 was constructed by K. Bertrand and is a derivative of pBR322 which has the BamHI-EcoRI fragment from pBR322 replaced with a 204-base pair (bp) Hasill fragment that contains the lac operator and promoter (personal communication). Both the BamHI and EcoRI sites were retained in pRZ4006 and transcription originating from the lac promoter is directed towards the BamHI site. The plasmid pNO1525 was constructed by digesting pRZ4006 with BamHI, treatment with DNA polymerase I and ligation with the Hasill fragment containing the L10 gene purified from λrh\*18. The resulting plasmid places the synthesis of plasmid-encoded L10 under the control of the lac regulatory elements. Plasmid pFM20 was constructed by ligating PxII-digested pBR322 to a PxII fragment (nucleotides 873-1,795) derived from λrh\*18. Plasmid pFM20 contains the β promoter and a portion of the L10 gene fused in phase' to the β-lactamase gene (bla) contained on pBR322 (ref. 24). Plasmid pGBΔBR2 was constructed by G. Barry and is described in ref. 17. The site for repressor action by L10 is indicated by a thick bar below the restriction enzyme site arrows.

 Table 1 Relative synthesis rates of proteins in the presence of L10

 Protein synthesized

 Template
 L10\*
 L7/L12
 Control protein

 Artical 18
 0.25
 0.38
 0.92

 HowdIII (1,700 bp)
 —
 1.05
 —

0 14

0.29\* 0.53\*

pNO1525

pFM20

. λ<del>β trp lac</del>1†

Proteins were synthesized as described in Fig. 2 legend in the presence and absence of 1.9  $\mu$ M L10. Synthesis of L10 and L7/L12 from  $\lambda nf^{41}8$  and synthesis of L10 from pNO1525 were determined by immunoprecipitation followed by gel electrophoresis. Synthesis of all other proteins were determined directly by gel electrophoresis. Fluorograms made using prefisshed film were scanned with a Joyce-Loebi densitometer. Ratios of amounts of synthesis obtained in the presence of L10 to synthesis obtained in its absence are shown Proteins listed as 'control' proteins for each template are:  $\lambda nf^{41}8$ ,  $\beta B'$ ; pNO1525,  $\beta$ -lactamase;  $\lambda B$ -try-lac1,  $\beta$ -galactosidsse; pFM20, a 35,000-molecular weight  $\beta$ -lactamase-L1 fusion protein containing the N-terminus of  $\beta$ -lactamase and the C-terminus of L1.

\*For  $\lambda\beta$ -trp-lac1 and pFM20, synthesis of the L10-trpB and L10- $\beta$ -lactamase fusion proteins was measured. The proteins of molecular weights 57,000 and 13,000 respectively were identified using anti-L10 antisera.

† $\lambda\beta$ -trp-lec1 was constructed by H de Boer by fusing the HmdIII-EcoRI fragment of  $\lambda$ rrf\*18 containing the  $\beta$  promoter (nucleotides 280-2, 154 in Fig. 1) to the left arm of HindIII-digested 'trp-lec' phage  $\lambda$  RS205-7 and to the right arm of EcoRI-digested  $\lambda$  phage 459. This method has been described for other ribosomal promoters\*7. The HindIII cleavage site in the trpB gene\*\* is in the same reading frame as the HindIII site in the L10 gene.

mRNA but had "little or no effect" on L7/L12 synthesis. We obtained maximal inhibition of L7/L12 synthesis by incubating the reaction mixtures at 30 °C. At 37 °C, our results were more similar to those of Brot et al., showing only 20–30% maximal specific inhibition of L7/L12 synthesis by L10 (data not shown). We believe that our results obtained in vitro at 30 °C reflect regulation in vivo because of the following results in vivo.

A complex containing four molecules of L7/L12 and one molecule of L10, which is formed on or off the ribosome <sup>14,15</sup>, will inhibit the *in vitro* synthesis of both L10 and L7/L12 by ~50% <sup>16</sup>. We have isolated the L10-L7/L12 complex and found its *in vitro* regulatory properties identical to those of L10 alone (data not shown). Thus, the inhibition of synthesis of L10 and L7/L12 by the complex must be due to the L10 within it. The same conclusion was reached by M. Johnson, T. Christensen and N. P. Fiil (personal communication). Interaction of L10 with the target on the mRNA seems to involve the part of L10 which interacts with 23S rRNA, and not the part that interacts with the four molecules of L7/L12.

To confirm that the regulatory properties observed for L10 in vitro reflect its regulatory role in vivo, the effect of over-

Fig. 2 Inhibition of in vitro synthesis of L10 and L7/L12 by addition of purified L10, L10 purified by phosphocellulose chromatography25 starting with a 1 M NH<sub>4</sub>Cl/50% EtOH wash of 70S ribosomes depleted of L7/L12 (ref. 26). L10 was stored in 6 M urea, 50 mM methylamine phosphate pH 6.5 at -70 °C. For use in in vitro experiments an aliquot of L10 (0.72 μg ml<sup>-1</sup>) was supplemented with bovine serum albumin (BSA; 1.0 mg ml and dialysed first against 0.5 M KCl/4 M urea/2 mM potassium phosphate pH 6.5/1 mM dithiothreitol and then against KUP buffer (1.0 M KCI/1 M urea/2 mM potassium phos-phate pH 6.5/1 mM dithiothreitol). In vitro reaction mixtures (40 µl) received either 2 μl of the resulting L10 solution (lanes 2, 4, 6) or 2 µl of

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KUP buffer containing 1 mg ml<sup>-1</sup> BSA (lanes 1, 3, 5). Proteins were synthesized in the presence of <sup>35</sup>S-methionine using 0.0017 μM λrif<sup>d</sup>18 DNA (lanes 1-4) or 0.009 µM HindIII 1,700-bp fragment (lanes 5, 6) as template, as described previously<sup>19</sup> except that incubation was for 2 h at 30 °C. The HindIII 1,700-bp fragment was prepared from pNO2020 (see Fig. 1). After incubations were completed, L10 was added to samples 1, 3 and 5. Reaction mixtures were analysed either directly by gel electrophoresis on a 13% polyacrylamide-SDS gel (lanes 5, 6) or by immunoprecipitation using rabbit anti-L10 antisera (lanes 1, 2) or anti-L7/L12 antisera (lanes 3, 4) followed by gel electrophoresis and fluorography19. A fluorogram is

production of L10 in vivo was examined. The HaeIII restriction enzyme fragment that contains the entire L10 gene and a small portion of the L7/L12 gene was inserted into a plasmid vector containing the lac operator and promoter so that synthesis of L10 was controlled by lac regulatory elements (pNO1525; see Fig. 1). The synthesis of L10 was stimulated by the addition of isopropyl thio- $\beta$ -galactoside, an inducer of the *lac* operon, in a strain which harbours the recombinant plasmid, and the synthesis rates of other individual proteins were then analysed.

The overproduction of plasmid-encoded L10 caused a marked inhibition of L7/L12 synthesis, and presumably chromosome-directed L10 synthesis, but had no effect on  $\beta$  and

Table 2 Relative synthesis rates of proteins after induction by isopropyl thio-βgalactoside or L-arabinose

	Strains					
	N02396	N02455				
Protein	(carries pN01525)	(carries pGB DBR2)				
S3	1.05	0.91				
S4	1.00	1.05				
S10	0.81	1.15				
L1	1.05	1.05				
L2	0.96	0.90				
L4	1.00	1.17				
L7/L12	0.26*	8.9*				
L10	1.80*	0.97				
$\beta + \beta'$	0.85	0.98				

For labelling experiments, cells were grown in a synthetic minimal medium24 supplemented with 0.4% glycerol, all amino acids (except methionine and lysine) at 50 µg ml<sup>-1</sup> and thiamine at 2 µg ml<sup>-1</sup>. Cells were grown at 37 °C to 2 × 10<sup>8</sup> per ml. Samples of cells were pulse-labelled with <sup>3</sup>H-lysine (646 pmol ml<sup>-1</sup>; 80.5 Ci mmol<sup>-1</sup>) for 1 min followed by a 1-min 'chase' with excess nonradioactive lysine. Cells were labelled before (control cells) and 10 min after addition of isopropyl thio-β-galactoside to 1 mM or 0.2% L-arabinose, depending on the strain used (experimental cells). Cells were rapidly chilled and then mixed with a suitable amount of <sup>14</sup>C-lysine-labelled carrier cells. <sup>14</sup>C-labelled cells were prepared by growing cells in the presence of <sup>14</sup>C-lysine for several generations. Ribosomal proteins were extracted and separated by two-dimensional gel electrophoresis<sup>30</sup>. The  $\beta$  and  $\beta'$  subunits were resolved from other proteins by SDS slab gel electrophoresis<sup>31</sup>. After staining the gels, the spots corresponding to the indicated ribosomal proteins were extracted, the gels oxidized with a Packard sample oxidizer and <sup>3</sup>H and <sup>14</sup>C measured separately. The <sup>3</sup>H/<sup>14</sup>C ratio in each protein in the experimental cells was then compared with that in control cells using  $[^3H/^{14}C]$  in protein i in experimental cells the expression:

[3H/14C] in protein i in control cells

The ratios obtained were then normalized to the ratio obtained for total cellular protein. Values given are the average of two independent experiments.

\* Values significantly different from 1.0.

β' synthesis (Table 2). The inhibition of L7/L12 synthesis was due only to L10 overproduction because no such inhibition was observed when the strain carried a deletion within the plasmidencoded L10 gene (data not shown). To test for a possible in vivo regulatory role of L7/L12, we used plasmid pGBΔBR2 which has L7/L12 gene expression under control of ara regulatory elements17. Addition of L-arabinose to a strain harbouring the hybrid plasmid caused an eight- to ninefold increase in the rate of L7/L12 synthesis but did not affect that of L10 or  $\beta$  and  $\beta'$ (Table 2). (We and others<sup>5</sup>, observed that in the in vitro system, purified L7/L12 does not inhibit synthesis of L10 or of L7/L12. We therefore conclude that L10 and L7/L12 are in a translational unit regulated by L10.)

How might L7/L12 synthesis be repressed by L10 acting at the L10 translational start site? One possible explanation is that efficient translation of L7/L12 mRNA requires the translation of the preceding L10 mRNA and that the inhibition of L7/L12 synthesis by L10 is a consequence of the inhibition of L10 synthesis by L10. Such 'translational coupling' has been observed in both the trp operon 18 and the L11 ribosomal protein operon19. Ribosomal proteins seem to be synthesized from other operons in units of translational regulation with the feedback repressors acting at single mRNA target sites. We have previously suggested a mechanism of sequential mRNA translation to account for the co-regulation and equimolar synthesis of ribosomal proteins in these regulatory units19. L7/L12 differs from other ribosomal proteins in that its synthesis is at least four times faster. We suggest that translation of L10 mRNA by a ribosome 'opens' the structure of L7/L12 mRNA initiation site. allowing other ribosomes to initiate the translation of L7/L12 at least four times as efficiently as that of L10 mRNA. We might expect that, without independent feedback regulation of L7/L12 synthesis by itself, more than the four copies of L7/L12 needed per ribosome might always be synthesized. In fact there are reports that, unlike other ribosomal proteins, significant amounts of L7/L12 exist in cells as free proteins 20,21. Regardless of the exact stoichiometry of the rates of L7/L12 synthesis relative to L10, the present work suggests a mechanism for the co-regulation of the synthesis of L7/L12 and L10.

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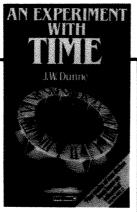


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## **BOOK REVIEWS**

## Eureka! No mystique to creative thinking

## Stuart Sutherland

EVERYBODY, from Arthur Koestler to Edward de Bono, knows that there is something very mysterious about creative thinking. To achieve it one must allow one's unconscious mind to work in unusual ways in order to produce that sudden insight that prompted Archimedes to leap from his bath and startle the citizens of Syracuse by running naked through the streets. Unfortunately, the fact that these thought processes cannot be specified has not stopped Arthur Koestler and others of his ilk from writing about them at length and giving them a variety of curious names such as biassociative, Janusian, paleological, homeospatial, divergent and lateral thought. In The Mind's Best Work, David Perkins debunks the mystery of creation. Just as a great athlete uses the same muscles as the puniest individual, so the great creators use the same mundane thought processes that mediate everyday existence. To prove his point he draws not merely on the intellectual biographies of such men as Darwin and Coleridge, but on a variety of ingenious experiments undertaken by himself and others, backed up by a series of demonstrations that the reader can try out on himself.

Two of the thought processes common in creative thinking are recognition and noticing. In seeking for a difficult proof, the mathematician may recognize a similarity between the structure of the problem and that of one for which there is an existing proof, thus suggesting a method of approaching the problem. Noticing occurs when some relevant feature of a problem is detected without any conscious attempt to discover it. Just as a woman interested in fashion may notice a flaw in a new costume, so the poet may notice that a word or phrase he has written is weak. What is noticed depends very much on one's interests and knowledge. The mind has a remarkable power to recover from memory items that satisfy very diverse criteria. Anyone can recover from memory the name of a food that is white and soft and whose name begins with an "s", even though he has never been confronted with this combination of criteria before. In the same way someone working on a new problem with particular criteria for solution may discover an answer that will be regarded as original.

Perkins's own research, in which he obtained protocols of subjects' thought

The Mind's Best Work. By D.N. Perkins. Pp.368. ISBN 0-674-57627-6. (Harvard University Press: 1981.) \$18.50, £12.95.

processes whilst solving problems, suggests that the mind rarely takes great leaps. It proceeds in a step-by-step fashion through successive acts of recognition and noticing which lead towards a solution. Where a sudden leap is taken, it is because through practice on a particular type of problem someone has learned to recognize a larger pattern that is of significance.

Moreover, creative thought is not wayward — there is a reason for each step taken. The reason can usually be articulated, but not always. In everyday life we may recognize that a friend is looking morose without being able to specify what aspect of his demeanour the judgement is based upon. All creative activity involves noticing flaws, and Perkins found that good painters and poets were better than poor ones at articulating what was wrong with an unfinished work. A gut feeling that something is wrong is less help in putting it right than an ability to specify the nature of the flaw and thus to establish criteria for how to go about improving it.

The role of incubation in the unconscious mind has, according to Perkins, been overplayed. Where it helps - and there is evidence to suggest that it rarely does - it probably does so for one of two reasons. First, one of the main obstacles to solving a problem is the difficulty in giving up a particular approach which is not paying off. A respite from thinking about a problem may make it easier to try a new approach. Second, whilst someone is thinking of something else, he may be lucky enough to encounter a useful hint that could not readily be obtained in the course of a frontal attack. In just this way, Malthus's essay on population gave Darwin the clue he needed to suggest the survival of the fittest as the selective mechanism in evolution. Laboratory experiments clearly establish the importance of such hints, many of which are used by subjects without their being aware that any hint has been

Creativity in a particular field depends, of course, on a thorough knowledge of it and on practice in existing techniques.

Abstraction plays an important role people must learn that a heuristic that has been found to solve one problem may be used to solve similar problems. Again, the more the heuristics are articulated the more helpful they are. In one experiment, a group of students in a class on integral calculus were shown how the use of five heuristics could help in solving sample problems. A second group were shown how to solve the same initial set of problems, but no effort was made to emphasize the generality of the heuristics used. In testing on further problems, the former group solved twice as many as the latter

In considering the question of what makes someone creative. Perkins points out that the obvious answer is usually ignored. An important element in creativity is the desire to be creative; one can set oneself to reject the obvious and to search for new solutions to a problem. The standards people set themselves in solving problems can be all important for the solutions they produce. Subjects urged to suggest "a good title for a novel" whose plot they had been given gave solutions that were markedly inferior to those produced by subjects urged to provide "an imaginative, creative or unusual title for this plot". There is also evidence that artists and poets think critically about their own work and a willingness to accept criticism from others correlates with the quality of their achievement. Art students who were open to criticism were found a few years later to be more successful than those who rejected it, though this particular experiment may only show that an artist who wants to please his public will sell more paintings than one who does not care what the public thinks. Because of the importance of self-criticism, geniuses are not necessarily fluent: Beethoven composed with painful deliberation. One of Perkins's more curious findings is that the average rate of composition of pentameters by both good and bad poets is half a line a minute and he claims that even Byron, reputedly one of the most fluent poets, did not exceed this rate in writing The Corsair. But if fluency is not a necessity for creation, luck often is. The man must be nicely matched to the problem. Einstein's intolerance for contradictions motivated the special theory of relativity, but the very same trait led him to waste his later years seeking an alternative to quantum theory.

It has only been possible here to sketch the nature of Dr Perkins's approach to creativity. He develops the points I have made in much more detail and makes many other illuminating suggestions. Although scholarly, his book is intended for the general public and is easy to read; it has only two faults — a tendency to write down to the reader and the inclusion of some singularly unattractive line drawings, which have little relevance to the text and serve to demonstrate only that not all artists are creative. He is of course aware

that reducing the thought processes of creativity to those employed in everyday life does not explain the nature of those thought processes. But this reduction is a good beginning if only because it frees psychologists to get on with the task of explaining ordinary thought processes, and may help to stop other authors from continuing the existing tradition of producing turgid and obscure tomes on the mysteries of creation.

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

## Contradictions in theoretical chemistry

H.C. Longuet-Higgins

Chemistry, Quantum Mechanics and Reductionism. By Hans Primas. Pp.451. ISBN 3-540-10696-0. (Springer-Verlag: 1981.) DM 68, \$32.40.

"THE purpose of this book" writes Professor Primas "is to provide a deeper insight into the modern theories of molecular matter. It . . . is not meant to be a textbook; in many respects it has complementary goals. For good and bad reasons, most textbooks ignore the historical and philosophical aspects [of theoretical chemistry] and go ahead on the basis of crude simplifications; many even lie like the Devil and do not shrink from naive indoctrination".

Remarks such as these are well calculated, as the author intends, to ruffle the placid waters of theoretical chemistry. It is difficult enough, in all conscience, to explain the technicalities of quantum chemistry to lively young minds without having one's authority undermined by pronouncements such as "the theoretical basis of chemistry and biology is not safely in our hands". From a scientist of less achievement than Professor Primas such an assertion might well be greeted with open hostility in the community of theoretical chemists. How can it be reconciled with our evident ability to compute, with greater and greater accuracy, the dissociation energies, dipole moments, force constants and other properties of molecules? If the Schrödinger equation is not good enough for Professor Primas, has he a better one to propose?

Such a reaction to the opening pages of Chemistry, Quantum Mechanics and Reductionism would entirely miss the author's point. Anyone who has studied and taught theoretical chemistry for any length of time will know how difficult it is

The latest supplement to A Bibliography of Ab Initio Molecular Wave Functions by W.G. Richards et al. has just been published by Oxford University Press. Covering the years 1978–1980, the book costs £35.

to reconcile, in his own mind and the minds of his students, the elusive properties of sub-atomic particles with the evident permanence of large molecules and crystals. There is an almost irresistible temptation to suppose, and to imply, that there is one set of laws for the very small and another for the large — a proposition which is in flat contradiction with the orthodox interpretation of quantum mechanics. Professor Primas asserts that

The mode of activity exhibited by contemporary science strikingly reminds one of what Shapiro (1965) has called the paranoid style. It is characteristic of this style that bridges between related problems are broken down so that things remain neatly and rigidly separated. Scientists who cultivate a paranoid research style are usually extremely acute and intense, show an exorbitant respect for compartmentalisation and computers, and firmly demand complete autonomy for their narrowly fixed ideas . . . The separation of philosophy and science has led to the so-called restricted world view and to the blindness of many experts who are entirely unaware of the abstract and isolating nature of modern science.

No, these are not the ravings of a iconoclast; in this long and fascinating book (it runs to over 400 pages) the author reveals a deep understanding and appreciation of the most difficult problems of quantum mechanics and of the acute paradoxes associated with its interpretation - or rather, with those alternative interpretations which do not violate experimental fact. He reveals himself as a master not only of the mathematics but also of the philosophy of science, and as a man who cares passionately about intellectual integrity, in the widest possible sense of that word. The book is uncompromisingly difficult, in an area where compromise would be unforgivable; but it is spiced with good plain questions of an acutely embarrassing kind: "What is quantum mechanics about?" "What is a classical description?" "Do isolated quantal systems exist at all?" "Does quantum mechanics apply to large molecular systems?" and so on. I wonder how

most theoretical chemists would answer these questions if some bright student were tiresome enough to ask them!

Chemistry, Quantum Mechanics and Reductionism is Professor Primas's own very positive contribution to the solution of some of these problems. The bestknown fully-developed interpretation of quantum mechanics is that of von Neumann, London and Bauer, and from this Professor Primas wants to remove just one unproved axiom, namely the universal validity of the superposition principle. He develops an alternative interpretation of his own, based on sophisticated algebraic concepts, of which the most important is that of a "W\*-algebra". To master his ideas would obviously take much time and thought, but they are logically compatible (as the orthodox interpretation is not) with the attribution of "classical" properties to molecules - an assumption which all theoretical chemists use without apology when referring to the charge, the mass or the structure of an ordinary molecule. The book may be difficult, but it is quite unusually honest and thoughtful, and is likely to prove an invaluable antidote to the narrowness of outlook which is so often apparent in chemical theorizing.

H.C. Longuet-Higgins is Royal Society Research Professor at the University of Sussex.

## Crucial colonization

**Barry Cox** 

The Terrestrial Environment and the Origin of Land Vertebrates. The Systematics Association Special Volume, No. 15. Edited by A.L. Panchen. Pp.633. ISBN 0-12-544780-9. (Academic: 1981.) £38, \$91.50.

The earliest known tetrapods are the ichthyostegid amphibians, now known from Late Devonian rocks in both Greenland and Australia. From this beginning sprang a variety of larger "labyrinthodont" amphibians, apparently ancestral to the reptiles, as well as the smaller "lepospondyl" amphibians. The transformation of our interpretation of palaeogeography, as well as progress in such fields as Palaeozoic floras and both invertebrate and vertebrate faunas, makes this a timely volume. The 20 papers were originally given at a symposium in Newcastle upon Tyne in April 1979, which was organized by Alec Panchen, editor of

Two of the papers are concerned with the geography of the world in which the first tetrapods evolved. Tarling analyses palaeomagnetic data and provides a series of continental reconstructions for the Early Devonian to Late Permian; he

suggests that China may have provided a link between Australia and Euramerica. The relationship between the changing Carboniferous geographies and the dispersal of early tetrapods is discussed by Johnson.

Two other papers deal with Palaeozoic botany. Dianne Edwards considers the appearance, habits and affinities of early vascular plants, but concludes sadly that little progress has been made in reconstructing the details of terrestrial plant communities of Devonian times, while Scott reviews the ecology of Late Palaeozoic floras, emphasizing the innovative effects of the evolution of arborescence and seed production. The only other non-vertebrate contribution is that by Rolfe, who describes the early invertebrate terrestrial faunas; these were dominated by arthropods, the larger of which, together with the amphibious eurypterids and scorpions, must have provided a food source for the earliest amphibians.

In one of the most interesting of the vertebrate papers, Thomson points out that there is little evidence that any of the Devonian lobe-finned fishes were primarily freshwater in habitat; he believes that the amphibians may have evolved from fish that still spent at least part of their lives in coastal lowland estuaries. But to which lobe-finned group did this fish belong? Opinions seem to be becoming more, not less, diverse. Though Rackoff's study of the fins of one osteolepid rhipidistian shows a number of close resemblances to the tetrapod limb, Janvier believes that the osteolepid pectoral girdle. though variable, could not have evolved into that of tetrapods. But an even more heretical phylogenetic idea also appeared at the symposium. Both Patterson, in an historical review of the problem of tetrapod origins, and Gardiner in a reappraisal of current evidence, suggest that the lungfish are the closest relatives of the tetrapods. The debate on this central issue promises to be interesting and fruitful.

In another morphological paper, Carroll notes the similarity between the hyomandibular of rhipidistians and that of early amphibians, and suggests that the bone retained a cheek-support function well into the Amphibia, only a few lines having independently transformed it into a sound-transmitting stapes.

Two early tetrapod faunas are reviewed. The new basal Late Carboniferous Scottish Cowdenbeath fauna is described by Smithson; it includes aquatic fish-eating and possibly herbivorous forms, as well as one genus that may have eaten terrestrial invertebrates, but provides few antecedents for later faunas. Andrew Milner makes a detailed analysis of the well-known, very diverse, later Carboniferous fauna of Nýfany, Czechoslovakia, and distinguishes its different components — open-water, shallow swamp-lake and

terrestrial-marginal endemics, as well as rarer elements from neighbouring environments.

Finally, a number of authors discuss the origin and classification of particular taxa—the anthracosaurian amphibians (Panchen), the Nectridea (Angela Milner), the Cotylosauria (Heaton), the Pelycosauria (Reisz) and the major amniote groups (Gaffney).

The contributions maintain a high

standard of interest and readability, and the volume is attractively produced; there are few typographical errors, and there is both an author and a subject index. It should serve for many years as both a benchmark of our knowledge, opinions and attitudes today, and as a source of references to the earlier literature.

Barry Cox is Professor of Zoology at King's College, University of London.

## Electron microscopy at the limits

David J. Smith

Experimental High-resolution Electron Microscopy. By J.C.H. Spence. Pp.420. ISBN 0-19-851365-8. (Clarendon/Oxford University Press: 1981.) £35, \$74.

THE technological revolution of the past decade has resulted in a concomitant improvement in the flexibility and usage of the commercially available electron microscope, not least in the direction of improved performance. Lattice and point resolutions in the region of 1Å and 3Å, respectively, have become standard. Hence, instead of high-resolution electron microscopy remaining an esoteric art, it is, in principle at least, now available to all those who can afford the admittedly considerable price of the latest instruments. That more scientists have not yet taken advantage of the opportunity to resolve physical details almost at the atomic level probably reflects the fact that recording a high-resolution micrograph, even with these advanced microscopes, is still difficult; moreover, the literature does not provide much guidance to the newcomer. This readable account of highresolution methodology thus represents a sorely needed addition to the field.

The book can be considered to consist of two parts. The first, comprised of Chapters 3 and 4, the first half of Chapter 5 and most of Chapter 6, provides a useful introduction to dynamical electron diffraction theory, setting out clearly most of the underlying concepts and in particular emphasizing the possible pitfalls of micrograph interpretation. Copious references have been included, making these chapters useful even to experienced workers. These sections are also carefully cross-referenced to the remainder of the book

On the experimental side, there are separate chapters on lenses and electron sources; much of the information here is tangential to the major theme of the book, and could well be omitted in a first reading. Nevertheless, these chapters represent valuable compilations of background material and, again, are well-referenced.

The remaining chapters deal with the practical problems involved in realizing

high-resolution micrographs, pointing out the requirements for knowledge of relevant electron-optical parameters, describing possible sources of interference and instability, and discussing operating techniques. These details will be of greatest value to the novice but many experienced workers will undoubtedly also find information of relevance to their research.

Despite the obvious attractions of the book, it is not free of some misleading or incorrect statements, and there are certainly strong arguments for re-ordering the subject matter. For example, the second half of Chapter 5, which briefly describes some applications, could well follow Chapter 10 on methods, and Chapters 1 and 2, which primarily deal with experimental details, would be better placed after the theoretical discussions of Chapter 6. The worst of the factual errors, presumably due to the publisher rather than the author, is located on the dust jacket where it is claimed that "Applications . . . are all described in full practical detail . . ." - this was manifestly not one of the author's objectives. It would also appear unlikely that any manufacturer of lathanum hexaboride cathodes would readily acquiesce to operation in vacuum levels of only "better than 10-5T" (p.262), and most users of field-emission sources would expect tip lifetimes of the order of several months, irrespective of operating voltage, rather than "about 80 hours" (p.263).

Such criticisms are obviously minor, and overall the author has produced a text which is far more than a "recipe book" for the beginner. He has evidently drawn on the wealth of knowledge of his many friends and colleagues in Oxford, Melbourne and Tempe, three of the leading centres in the field, as well as his own practical experience, and his book should become a valued guide and source of information for the practising microscopist.

David J. Smith is Director of the High Resolution Electron Microscope Project at Cambridge University.

## Fish-heads and breadcrumbs to feed the five thousand

John Rivers

Nutrition and Food Science: Present Knowledge and Utilization. Edited by Walter J. Santos et al. Three-volume set. Vol. 1, Food and Nutrition Programs and Policies, pp.850, ISBN 0-306-40342-0. Vol. 2, Nutrition Education, Food Science and Technology, pp.900, ISBN 0-306-40343-9. Vol. 3, Nutritional Biochemistry and Pathology, pp.758, ISBN 0-306-40344-7. (Plenum: 1980.) Vol. 1 \$75, £47.25; Vol. 2 \$79.50, £50.09; Vol. 3 \$69.50, £43.79. Three-volume set \$195, £122.88

Nutrition and Food Science is a weighty work: three volumes, 2,500 pages, 750,000 words. That is about as long as the Bible or the complete works of Shakespeare. It seems reasonable to ask if these three books measure up to such standards.

The answer, sadly, is no. These volumes will rarely be read, and even more rarely enjoyed. They cannot even be treated as a coherent work: they are a patchwork of 250 essays linked by the fact that their authors attended the Eleventh International Congress of Nutrition in Brazil, 1978.

The editors have given each volume a subtitle and subdivided each volume, presumably to impose some order on them, but this attempt fails. For example the title for Vol. 2, Nutrition Education, Food Science, and Technology, is in itself a portmanteau. Fittingly so, since the volume is further subdivided into four parts — Animal and Vegetable Resources for Human Feeding, Food Science and Technology, Research in Food Nutrition (sic) and Nutrition Education — and since national nutrition surveys are put in Vol. 2 even though Vol. 1 seems more appropriate.

In this electronic age the international congress is becoming an anachronism. In small fields, especially fast-moving ones, it is still a feasible and important way of communicating. But all too often congresses are only "holidays for the boys". The cliché that the best science is discussed in the bar is actually a two-edged one.

Some time ago the Economist magazine, in an article on scientific research, noted that, of the 32 participants at an international physics conference in 1927, a third were, or were to be Nobel Laureates. An equivalent meeting in 1980 had 800 participants, no Nobel prizewinners and only routine investigations were reported. The Economist should have dissected nutrition. The triennial International Nutrition Congresses have doubled in size about every ten years. The science they represent grows only one-quarter as fast, so necessarily the congresses have become less and less important as a legitimate part of scientific communication, and more and more a social event.

At the Eleventh Congress, 5,000

participants gathered together in Brazil and seem to have achieved nothing: were all those journeys really necessary? Like a somewhat more famous gathering of 5,000 they subsisted on a very meagre diet. But if a miraculous transformation of the loaves and fishes of their intellects occurred, then it must be the baskets of leftover rubbish that have been published for posterity.

For that is what this work is about. It is a three-volume commemoration of an event. There are some good, original papers, such as Jul's study on dairy farming in Calcutta, but they are few, and will probably be lost in the mound of fish-heads and breadcrumbs. A mound of papers in which nothing much is said, in which most tables

and graphs seem to be referred back to previous publications, in which representatives of commercial organizations are simply reporting the use of their products, and a collection which overall could have done with refereeing and extensive subediting.

Nutrition and Food Science is a reverberating cry for something to be done to stem the flow of paper that is dignified by the appellation "scientific literature". This is neither science nor literature.

John Rivers is a Lecturer in the Department of Human Nutrition at the London School of Hygiene and Tropical Medicine, University of London

## Depth in stability for the cognoscenti

P.H. Roberts

Hydrodynamic Stability. By P.G. Drazin and W.H. Reid, Pp.525. ISBN 0-521-22798-4. (Cambridge University Press: 1981.) £35, \$77.

"Not every solution of the equations of motion, even if it is exact, can actually occur in nature. The flows that occur in nature must not only obey the equations of fluid dynamics, but also be stable". The significance of this apt quotation from Landau and Lifshitz with which the authors of Hydrodynamic Stability open their book is evident from the scarcity in nature of simple orderly ("laminar") motions and the abundance of irregular ("turbulent") flows. The transition from the former to the latter starts with the instability of the laminar flow, a process that can be studied in controlled laboratory conditions and, with comparative ease, by theoreticians.

The achievements of stability theorists are now so extensive that the authors had to make hard decisions in limiting their text to even 525 pages. They have opted for depth, rather than breadth, of treatment, and paid particular attention to classic situations such as Bénard convection, circular (Couette) flow and plane parallel flow.

A consideration in the authors' choice of topics was probably the existence of Chandrasekhar's classic work, Hydrodynamic and Hydromagnetic Stability (Clarendon Press, 1961). Wonderful though Chandrasekhar's treatise is, it is sometimes felt today that it contains a number of lacunae: it overstresses variational methods; it ignores a useful technique (asymptotic analysis); it omits one of the oldest and possibly the mathematically most interesting of all linear stability problems, that raised by

plane Poiseuille flow; and it says little about non-linear stability. None of these points would have been fair criticism in 1961 when the book was published, but today many specialists will welcome Drazin and Reid's account, if for no better reason than because it successfully redresses the balance.

The authors are of course well known, particularly for their work on parallel flows, and it is here that readers will discover the most penetrating discussions, and the deepest and most elegant mathematics. Indeed, some may wonder whether the authors' enthusiasm has carried them too far, and whether all of the sophisticated theoretical weapons displayed are essential to their own armouries. This space might have been better used in expounding non-linear theory still further, or in providing deeper discussions of baroclinic instabilities, another subject that has exploded since Chandrasekhar wrote his book. Most readers will find the discussion of pinch instabilities frustratingly brief.

These are minor reproaches only. The work is undeniably of high scholarship, consummate accuracy and penetrating insight. Teachers of graduate courses will find the problems at the end of each chapter imaginative, but difficult: solutions can be obtained by application to either author. All specialists in stability theory will be happy that two such authorities have found the time, and spared so few pains, to produce a work of such excellence.

P.H. Roberts is Professor of Applied Mathematics at the University of Newcastle upon Tyne.



## **Energy: Present and Future Options**

edited by D. Merrick, National Coal Board, Coal Research Establishment, Cheltenham.

and R. Marshall, Department of Physics, The University, Keele This volume reviews a number of options for future energy supplies, and possible technologies for conversion and use. The options selected are those which are generally expected to become important in the future, as supplies of oil and natural gas become scarcer and more expensive. A companion volume will review those technologies not covered here.

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The ideal candidate will have a post graduate qualification and experience in the area of recombinant DNA research.

The tasks will include the acquisition, execution/management of research projects, including the presentation of results. Creativity in the development of project concepts and willingness to work in interdisciplinary teams are essential advantages.

**Application:** 

Candidates should call us (0611/79082686 or 79082478) or send us their brief application. They will receive our answer by return.

Battelle-Institut e.V., Postfach 900160, Am Römerhof 35, 6000 Frankfurt am Main 90





## Chief, Invertebrate & Marine Plants Division

Salary: \$37,703 - \$47,448 (under review)

Ref. No.: 81-PSCH-DFO-12

Department of Fisheries & Oceans Halifax, Nova Scotia

Plans, directs and manages the Invertebrates and Marine Plants Division; organizes and administers activities aimed at maintaining the welfare of marine invertebrates and plant resources and maximizing continuing biological production from stocks; co-ordinates work of the Division with that of industry, provincial departments and other units within the Fisheries organization.

### Qualifications

Either graduation with an acceptable doctoral degree from a recognized university in Biology or an equivalency. (Equivalency refers to graduation with an acceptable Master's Degree or an acceptable Bachelor's Degree from a recognized university in Marine Biology coupled with acceptable research training and experience in which the capability has been clearly demonstrated to do independent research equivalent to that required for a doctoral degree.): evidence of authorship of published or unpublished reports or papers resulting from research in the field of fisheries; and recognition by the scientific community of achievement in fisheries research; experience in carrying out research projects related to marine invertebrates and plant resources and in the management of research resources, including supervision and financial budgeting and control.

Knowledge of the English language is essential.

Clearance No.: 331-259-001

Additional job information is available by writing to the address below:

Toute information relative à ce concours est disponible en français et peut être obtenue en écrivant à l'adresse

### How to apply

Send your application form and/or résumé to: Mrs. B.J. Firth, Staffing Officer Public Service Commission of Canada 4th Floor Royal Bank Building 5161 George Street Halifax, N.S. B3J 1M8 902-426-5878 Closing date, December 31, 1981

Please quote the applicable reference number at all times

(NW048)A

## **UNIVERSITY OF MIAMI** MARINE SCIENCE

The University of Miami is searching for a Dean who will serve as the Director of its oceanographic and atmospheric Institution which has 86 full-time faculty, 162 graduate and 305 undergraduate students, and a \$14 million budget. The main function of this position is to provide leadership to a thriving, multidisciplinary community of oceanographic researchers and students, including biological, chemical, physical and geological oceanographers, ocean engineers, atmospheric scientists and specialists in marine affairs. The person who directs this Institution will be expected to promote its aims and provide liaison with federal agencies as well as private foundations, and to encourage new programs to meet the needs of both developed and developing nations, especially those of the Caribbean and South America

Applications, including a current professional résumé, nominations and further information should be sent of Dr. Norman G. Einspruch, Chairman of the RSMAS Dean Search Committee, University of Miami, School of Engineering and Architecture, PO Box 248294, Coral Gables, Florida 33124. Nominations and applications desired by December 15, 1981. Position will remain open until filled.

An Equal Opportunity/Affirmative Action Employer. (NW013)A

## **Executive Director**

## **HUNTSMAN MARINE LABORATORY** St. Andrews, New Brunswick

Applications are invited for the above position.

Founded in 1970, the HML is operated by a consortium of universities, government departments and private sector agencies at a base for research and teaching in the marine sciences. The HML also undertakes contract research in several areas of marine

Salary and starting date negotiable, but hopefully the post will be occupied by July 1, 1982. Canadian immigration regulations require that only Canadians or landed immigrants need apply for

Applicants should submit a curriculum vitae and two letters of reference to Dr W.C. Leggett, President, Huntsman Marine Laboratory, St. Andrews, N.B. E0G 2X0. Deadline for receipt of documents January 1, 1982. INWOS21A **Department of Chemistry** 

# POSTDOCTORAL RESEARCH POSITIONS

Applications are invited from well qualified candidates for one or two year appointments as Postdoctoral Research Fellows in the following fields:

Analytical chemistry; Atmospheric photochemistry; Bioinorganic chemistry; Catalysis using transition metal complexes; Chemistry atomic and solid state physics studies with Muon Spin Rotation (SR) at TRIUMF: Circular polarization in protein fluorescence; Collision theory of non-sperical molecules; Development of new synthetic methods in organic chemistry; Electron energy loss spectroscopy and coincidence experiments; Electron paramagnetic, ENDOR, Ferrocene derivatives, NQR, Mössbauer, microwave and electronic spectroscopies; Flash photolysis and kinetic spectroscopy; Fluorocarbon chemistry; Fourier transform ion cyclotron resonance spectroscopy; Homogeneous and heterogeneous catalysis; Immunochemistry of polysaccharides; Inorganic fluorine chemistry and studies in highly acidic media; Kinetic theory of gases applied to astronomy and space science; Laser bleaching studies: Magnetic resonance studies of ordered fluids including liquid crystals and biomembranes; Marine natural products; Mechanism of the intercalation of layered structures; Muonium chemistry; Non-linear optical effects in organic solids at low temperature; Nuclear magnetic resonance studies of molecular motion and of chemical shifts in solids; Organic and organometallic ion-molecule chemistry; Organic solid state reactions; Oxidation, use of coenzyme models, general acid catalysis; Phosphazene and thiazyl chemistry; Photoelectron spectroscopy including LEED. Auger and ESCA studies; Physical biochemistry of cell surfaces; Physical chemistry of biological morphogenesis; Physical chemistry of proteins; Quantum chemistry and molecular physics; Radiation chemistry and solvated electron studies; Sub-Doppler laser electronic spectroscopy: Synthesis of natural products; Synthesis of potential antiviral and antitumor compounds; Synthesis and study of organic metals; Theory of molecular relaxation and transport properties; Whole cell NMR studies; X-ray diffraction

The minimum stipend for the first year will be \$14,000. A substantial number of Teaching Postdoctoral Fellowships, with a minimum stipend of \$17,245 are open to applicants who have recently graduated and have shown superior ability in research and scholarship.

Send curriculum vitae, transcripts, and letters from three referees to the undersigned, from whom further particulars may be obtained.



J. Trotter,
Professor and Acting Head,
Department of Chemistry,
2036 Main Hall,
UNIVERSITY OF BRITISH COLUMBIA
Vancouver, British Columbia,
CANADA V6T 1Y6

(NW046)A

## THE UNIVERSITY OF MELBOURNE CHAIR OF GENETICS

The Chair of Genetics is vacant following the resignation of Professor M J Whitten.

Applications are invited from distinguished geneticists, preferably interested in eukaryotic genetics.

Salary at present is \$A41,509 per

Further information about the position, including details of application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Registrar of the University or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square London WC1H 0PF. All correspondence (marked "Confidential") should be addressed to the Registrar, The University of Melbourne, Parkville, Victoria 3052, Australia.

Applications close 1 Feburary 1982. (9879)A

## UNIVERSITY OF NOTTINGHAM

Medical School

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

Applications are invited from those qualified in medicine, physiology, pharmacology or cognate disciplines for a

### LECTURESHIP

which arises from the appointment of Dr C A Marsden to a Wellcome Trust Senior Lectureship within the department.

Physiology and pharmacology are taught as basic medical sciences as part of an integrated curriculum for Part I of the Bachelor of Medical Sciences degree. An Honours option in Physiology and Pharmacology is available to medical students for Part II of the degree.

There are excellent facilities for research in human and other branches of physiology and pharmacology. The current research interests of the department include carciovascular and respiratory control mechanisms; effects of exercise and training on the cardiovascular system and on skeletal muscle; the measurement of daily physical activity; control of skeletal muscle including tremor and fatigue; central neurotransmitters; the measurement of drug effects and of drug metabolism; effects of hypoglycaemia.

Salary will be within the nonclinical scale for lecturers with membership of USS. The successful applicant will be expected to take up the appointment as soon as possible.

Those interested in this post are invited to write for details and a form of application to the Deputy Registrar, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, to whom completed applications should be returned by the 11th December, 1981. (9867)A

### GEOPHYSICAL FLUID DYNAMICIST/PHYSICAL OCEANOGRAPHER

Applications are solicited for a junior faculty position in ocean physics or dynamics to begin in the academic year 1982-83. Areas of interest to the Department include analytical, numerical and laboratory modeling of physical processes and phenomena in the sea.

Yale University is an equal opportunity/affirmative action employer and encourages women and members of minority groups to compete for this position.

Curriculum vitae, publications, and the names of three or more referees should be sent by 31 December 1981 to: Robert B Gordon, Chairman, Department of Geology and Geophysics, PO Box 6666, New Haven, CT06511. (NW1009)A

## ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

DEPARTMENT OF MEDICINE

Immunological Medicine Unit

Applications are invited for the post of RESEARCH FELLOW

(Senior Registrar Grade) in Immunological Medicine

This post is for a medical graduate at or approaching the senior registrar (salary on the scale £9,330 -£11,900 plus £527 London Allowance), although consideration will be given to clinicians at the registrar grade or to non-clinical scientists. The appointment, which is funded by the Medical Research Council, is for the period I April 1982 31 July 1984. The successful applicant will join a multidisciplinary team under the direction of Dr M B Pepys working on the acute phase plasma protein response to tissue injury and on amyloidosis. He/she will participate in a variety of projects including biochemistry, immunochemistry and clinical measurement of acute phase and amyloid proteins in the circulation and the tissues, and clinical and pathology experimental inflammatory lesions in relation to the acute phase response and amyloidosis. The work performed will be suitable for a higher degree (MD, PhD).

Further information available from Dr Pepys, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, Du Cane Road London W12 0HS, Tel: 01-740 8970/01-743 2030 ext 258.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 OHS quoting reference number 2/MBP. (9876)A

# Health Physics and Safety Officer

The Northern Division of the United Kingdom Atomic Energy Authority invites applications for the above position in a team with responsibility for the Authority's Risley and Culcheth sites.

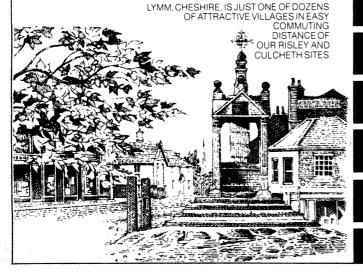
## THE WORK

The successful applicant will be responsible to the Head of the Health Physics and Safety Department for providing a complete personnel and environmental monitoring service with the support of a professional and technical team.

Duties will include assessing radiological and toxic hazards; framing operational instructions sufficient for the detailed observance of safe working practices; providing and operating a health physics advisory monitoring and emergency service; investigating radiological safety problems; advising and assisting in training programmes; representing the Department at appropriate internal and external health physics liaison meetings, and ensuring that statutory and mandatory records required for radiological protection are maintained.

## WHAT YOU NEED

Applicants should possess a relevant degree or corporate membership of an appropriate professional institution. Practical experience in the field of health physics would be an advantage.



## THE REWARDS

The position, as Professional and Technology Officer Grade 1, carries a salary ranging from £9,980 to £11,715 p.a. depending on qualifications and experience.

Conditions of employment and benefits are excellent, and include an attractive contributory superannuation scheme.

## THE PLACE

The Risley and Culcheth area features excellent schools, access to beautiful Cheshire countryside, first-class motorway links, easy connections with Manchester and Merseyside, and a wide variety of housing. Assistance with house purchase or single accommodation may be provided.

(9887)A

## SEND THE COUPON NOW. NO STAMP NEEDED.

Please send me an application form for the post of Health Physics and Safety Officer. S2401/N
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Post Code\_

Address

Mail this coupon to The Staff Officer, UK Atomic Energy Authority. Northern Division, FREEPOST, Fisley, Warrington WA3 6AT.

UK Atomic Energy Authority

## **Senior Biochemical Engineer**

At Merck Sharp & Dohme Research Laboratories, you'll find one of the largest R&D budgets in the pharmaceutical field and programs that are guided by management keenly aware of the needs of contemporary scientists and engineers. And that can make a difference in your career.

We're now seeking a Biochemical Engineer to serve as Chief Project Engineer for the start-up of our new Fermentation R&D Pilot Plant - the most sophisticated facility of its kind in the industry — and scale-up of new technology. This leadership role re-

quires 5-7 years experience in operations of pilot plant or production scale fermentors. Working knowledge of computers, and familiarity with modern process measurement instrumentation essential. A PhD (or equivalent) in Chemical Engineering or related field and background in biochemical process programs, fermentation process scale-up and research computer applications are desirable.

To join our aggressive R&D staff, send résumé to Ms. Johanna Y. Zeltner, Ref. NA-2,



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(NW047)A

## **FOREST ENTOMOLOGISTS AND** FOREST PATHOLOGIST

Vacancies exist in the Forestry Industry in the Republic of South Africa for the following permanent posts:

- 2 Graduate Entomologists
- 1 Technician with experience in Entomology
- 1 Graduate Pathologist

Appointment will be made on behalf of the Forestry Council by the Wattle Research Institute and appointees will be seconded to the Plant Protection Research Institute of the Department of Agriculture and

Location of the posts will be: One Entomologist and Technician with headquarters with the Wattle Research Institute, Pietermaritzburg, Natal.

One Entomologist with headquarters with the Department of Agriculture and Fisheries in Cape Town.

Pathologist with headquarters with the Plant Protection Research Institute in Pretoria, Transvaal.

Forestry experience will be a recommendation.

Duties will include monitoring of existing and potential forest insect pests and diseases on an individual and team basis under the direction of the Plant Protection Research Institute guided by the forestry Industry Technical Advisory Committee on Plant Protection.

Salary will be by negotiation.

Applications should be sumitted in the first instance to the Director, Wattle Research Institute, University of Natal, PO Box 375, Pietermaritzburg, 3200 Natal, Republic of South Africa, indicating present remuneration, and should be accompanied by curriculum vitae and the addresses of at least three referees. (9850)A

## UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

## SCIENTIFIC OFFICER/ POSTDOCTORAL WORKER

A vacancy has arisen for a graduate Scientific Officer or recently qualified postdoctoral worker to join a research group led by Professor Philip Cohen, who are studying the molecular mechanisms by which hormones regulate biosynthetic processes in mammalian cells. The person appointed should have a strong interest in instrumentation and will be responsible for the running of a protein sequencing laboratory comprising a Beckman 890C automated amino acid sequencer, high pressure liquid chromatography system and two amino acid analysers. The work is supported by a Programme Grant from the Medical Research Council, as well as by the British Diabetic Association, British Heart Foundation and Cancer Research Campaign.

Applications containing a full Curriculum Vitae, research interests and the names of three referees should be sent to the Personnel Office, University of Dundee, Dundee DD1 4HN, Scotland, UK, as soon as possible. The position can start immediately but candidates wishing to start at any time up to March 1st, 1982 will be considered. Please quote Ref. EST/59/81J.

UNIVERSITY OF **ALBERTA** Edmonton, Alberta, Canada T6G 2E3 DEPARTMENT OF GEOLOGY

The Geology Department has one permanent faculty position available July 1, 1982. We invite applications from qualified individuals for appointment at the

**ASSISTANT** 

or

## ASSOCIATE PROFESSOR LEVEL

in any of these areas: Geomorphology, Mathematical Geology, Engineering Geology, Process Sedimentology and Structural Geology. Preference will be given to those applicants who demonstrate an ability to pursue a vigorous research program applying modern concepts and techniques in solving geological problems. The candidate is expected to teach an undergraduate course in quantitative geomorphology, course(s) in his or her speciality, including if qualified, geostatistics. The position also involves super-vising Masters and PhD students. A PhD is required and salary is commensurate with education and ex-perience. Canadian citizens and permanent residents will be given preference.

Interested applicants should submit a résumé, publications and names and addresses of three referees to Dr NW Rutter, Chairman, Department of Geology, University of Alberta, Edmonton, Alberta, Canada T6G 2E3. Closing date for applications is February 15, 1982. applications is February 15, 1982. (NW063)A

## **UNIVERSITY OF** SUSSEX

Medical Research Council Cell Mutation Unit

## BIOCHEMIST **MOLECULAR BIOLOGIST**

Applications are invited for a Short-Term Non-Clinical post in this MRC Unit tenable for 3 to 5 years according to age and experience of the successful candidate. Candidates of immediately post-doctoral or equivalent status will be preferred, but well qualified candidates with less than 3 years postgraduate experience will also be considered

The successful candidate will work in a team studying the mechanisms of DNA repair in human cells. Some enzymological experience is desirable and the work will involve the application of recombinant DNA nology to human DNA repair genes.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information and an application form may be obtained from Dr A R Lehmann (tel: 0273 606755, ext 236). Application forms plus a full cv and the names of two professional referees should be sent to Mrs M Bunn, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG by January 31st (9870)A

## UNIVERSITY OF THE WEST INDIES — JAMAICA

Applications are invited for the following posts in the faculty of National Sciences:-

## LECTURER/ ASSISTANT LECTURER IN CHEMISTRY

Applicants with special interests in Applied Chemistry or Food Chem-istry will be given preference. The appointee will be expected to play a major role in the teaching and organisation of courses in one or more of the above fields and to assist in other areas of chemistry. Contributions to the research programme in whe Department will also be expected.

## LECTURER/ ASSISTANT LECTURER IN MATHEMATICS

(2 posts)

One post should be filled by a person with teaching and research experimence in Mathematical Statistics.

Salary scales: Lecturer 1\$15,090 J\$20,049 pa; Assistant Lecturer J\$13,308 — J\$14,088 pa (£1 sterling = J\$3.30). FSSU Study and Travel Grant. Unfurnished accommodation or housing allowance.

Detailed applications (2 copies), including curriculum vitae and naming 3 referees, should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Applicants resident in the UK should also send 1 copy to the Committee for Inter-national Cooperation in Higher The British Council Education, Higher Education Division, 90/91 Tottenham Court Road, London W1P 0DT. Further details are available from either address

(9865)A

### **UNIVERSITY OF** ARERDEEN

**DEPARTMENT OF** CHEMICAL PATHOLOGY

## POST-DOCTORAL **RESEARCH FELLOW**

(supported by The British Heart Foundation)

## (Re-advertisement)

Applications are invited for the above post, tenable for a maximum of three years, from protein biochemists to work on the structure of fibrin and fibrinogen in human atherosclerotic plaques, one aspect of a long-term research programme on atherosclerosis for which the Research Fellow will take major responsibility. Experience in modern methods of protein separation essential.

Salary on Range 1A Scale, starting point up to a maximum of £6,985 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen with whom application (2 copies) should be lodged by 4 December (9869)A

## SANDOZ RESEARCH INSTITUTE in Vienna, Austria

is one of three international research centres of the Swiss based pharmaceutical company SANDOZ. We have an opening for a young

## ORGANIC CHEMIST

who by preference should have experience in the chemistry of protecting groups and in handling labile substances. This scientist will work in the field of chemical synthesis of DNA and will have his own laboratory. He is expected to have particular interests in problems of molecular biology and genetics.

The successful candidate will find excellent working conditions in well equipped laboratories. In addition, the fostering of professional contacts, and participation in specialized training courses are actively encouraged. We offer a competitive salary and all fringe benefits associated with a leading international pharmaceutical company.

Removal expenses incurred by the successful candidate will be defrayed by the Institute.

Please send curriculum vitae, list of publications and the name of referees to



Dr N. Zacherl, Personnel Manager, SANDOZ FORSCHUNGSINSTITUT, **Brunner Strasse 59** A-1235 Vienna, Austria

(W494)A

## UNIVERSITY OF NEWCASTLE UPON TYNE

**FACULTY OF** ARICULTURE

### RESEARCH ASSOCIATE

Applications are invited for the temporary post of Research Associate in the Department of Agriculture for a period of three years to work on a project sponsored by the Ministry of Agriculture, Fisheries and Food. The work will involve the development of a system allowing dry sows to be group housed but individually rationed via an electric sow-activated concentrate dispenser. Sow behaviour will be studied and long term sow production monitored.

Candidates should have either postgraduate research experience and/or qualification, or hold a First or Second Class Honours Degree in Agriculture or a related subject. In the latter case there will be opportunity to register for a higher degree.

Starting salary will be up to £6,475 oa on the Range 1A scale (£6,070 £10,575 pa) according to age, qualifications and experience.

Further particulars may be obtained from the Deputy Registrar (FP), The University, 6 Kensington Terrace, Newcastle upon Tyne NEI 7RU, with whom applications (3 copies), together with the names and addresses of three referees should be lodged not later than 30th November 1981. Please quote reference N

(9861)A

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## IROLOGIST

Substantial investment in R & D, facilities and brainpower: these are the keys to the on-going success of our company, which specializes in biologicals. As the company continues with a vigorous expansion programme, new opportunities have been created in research, production and quality

If you hold a Ph. D. or an equivalent degree in virology or a related life science and have relevant post-graduate experience, this position offers an ideal opportunity for you to advance. Career options are wide and will largely depend on your individual strengths and interests.

Our search is for high-calibre talent who can hold their own in our research-oriented organization Salaries and benefits offered match the situation.

Please send your detailed resume to

IMMUNO AG Dr. St. Karwautz, Personnel Manager Industriestraße 72 A-1220 Vienna, Austria





## **OVERSEAS** EVELOPMEN

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## **Head of Research Station**

## **Zimbabwe**

DUTIES: Based at Chiredzi the appointee will assume responsibility for the Lowveld research stations, he will:

- be responsible to the Director for all staff, resources and planning/management of the entire Lowveld research programme, undertaking work on both large and small scale production of irrigated crops, excluding sugar cane. Currently the programme is concerned with cotton, wheat and coffee also maize, soyabeans, groundnuts and a range of horticultural and other crops.
- be responsible for assisting with the identification of a successor and for initiating training of this officer.

QUALIFICATIONS: Candidates, aged about 40 years, should have a degree in a relevant agricultural discipline and some 15 years experience in research tropical/sub tropical agricultural (including irrigation) preferably with part of this period spent in a control post. A postgraduate qualification is desirable although experience will take priority.

APPOINTMENT 3 years. Salary: In a range above £16,825 pa subject to UK income tax, plus a variable tax-free overseas allowance in range of £1,265 - £2,845.

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting ref. AH364/X (RC 213/136/08) stating post concerned, and giving details of age, qualifications and (9864)A experience to:



Recruitment Executive.

OVERSEAS DEVELOPMENT ADMINISTRATION,

Room 351. Abercrombie House.

Eaglesham Road, East Kilbride.

Glasgow G75 8EA.

## HELPING NATIONS HELP THEMSELVES

## **UNIVERSITY OF CAMBRIDGE**

INSTITUTE OF ASTRONOMY

## RESEARCH APPOINTMENTS IN **THEORETICAL ASTRONOMY**

Applications are invited for SERCsupported postdoctoral positions for research in theoretical astronomy. Appointments will be for up to 3 years. The salary scale (under review) is £6,475 (age 25) to £9,750 (age 33), plus USS benefits. The preferred starting date is 1 October 1982.

Applications should be sent to Professor M J Rees, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA by 31 January 1982, and applicants should themselves ask three referees to write to Professor Rees by the same date. (9873)A

## POSTDOCTORAL POSITION

to study

## CYTOGENETICS AND MODIFICATION OF THE C-BANDING PATTERNS OF RYE AND TRITICALE

Experience in the cytogenetics of cereal plants is required and preferably some interest in the use of electrophoretic analysis of cereal grain proteins in conjunction with cytogenetic studies. The appointment is initially for one year with possible extension to three years. Position opens immediately. Salary range \$14,220 to \$16,380 pa, according to experience.

Applications (naming three referees) to Dr EN Larter, Plant Science Department, University of Manitoba, Winnipeg, Manitoba, Canada R3T (NW065)A

## UNIVERSITY OF **QUEENSLAND** Australia

DEPARTMENT OF CHEMISTRY (PHYSICAL)

## RESEARCH ASSOCIATE

A postdoctoral position for collaboration with Prof L E Lyons, FAA, in work on solar cells with 11-VI semiconductors. One year from 1 January 1982 with possibility of renewal. Salary in range \$A17,083 to \$A19,072 plus 10% superannuation and one way fare for one person. (Quate Ref. 39881).

Applications should be forwarded to the Staff Officer, University of Queensland, St Lucia, Queensland, Australia 4067, with copy to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

(9872)A



Medical Research Council Centre

LABORATORY OF **MOLECULAR BIOLOGY** 

## **Postdoctoral Peptide** Chemist

Applications are invited for a shortterm Non-Clinical Scientific post in the Laboratory of Molecular Biology, tenable for two years in the first instance with possible extension. Candidates should be of postdoctoral or equivalent status.

The person appointed will join a group working on the synthesis and biology of immunogenic peptides. Previous experience in peptide synthesis by solid phase or solution methods would be an advantage but training will be given to a suitable candidate with relevant experience in other fields.

Salary will be at an appropriate point on the scales for University Non-Clinical Academic staff, currently in the range £7,290 to £9,750 per annum, depending upon background, qualifications and experience.

Further information is available from Dr R.C. Sheppard, Laboratory of Molecular Biology (Cambridge (0223) 248011).

Applications as soon as possible with curriculum vitae and names of two professional referees, and quoting reference No. PC/10 to: The Administrator, MRC Centre, University Medical School, Hills Road, Cambridge CB2 2QH

(9855)A

## **ASSISTANT PROFESSOR**

The Department of Biological Chemistry of the University of Illinois invites applications from candidates for the position of Assistant Professor beginning in the academic year 1982-83. The candidate is expected to develop a research program in any area of biochemistry that will complement the activities of the existing faculty. Candidates should possess a PhD degree with a strong chemical background, at least two years of postdoctoral experience, and published evidence of ability to design productive experiments in their intended area of biochemical research. Curriculum vitae and three supporting letters of recommendation should be sent to: Biological Chemistry Search Committee; University of Illinois Medical Center; 1853 W. Polk Street; Chicago, Illinois 60612. The University of Illinois is an Equal Opportunity — Affirmative Action

Opportunity — Affirmative Action Employer and encourages applications from women and minorities.

(NW066)A

## UNIVERSITY OF STOCKHOLM

DEPARTMENT OF ZOOLOGY

## ETHOLOGIST OR ECOLOGIST

Applications are invited for a position as "eo docent" (roughly equivalent to US assistant professor). The position is primarily intended for a person with several years of post-doctoral research experience.

The position is advertised for a period of three years, but is usually renewed for another three year period. Starting salary L 18 (Swedish crowns 107,088 per annum = roughly 19,000 US\$).

The position is a research and teaching position, and requires a minimum of 75 hours of lecturing per annum or equivalent supervision of graduate student research, some administrative duties, and a vigorous research programme. Applicants are selected primarily on the basis of their scientific publications, but teaching experience is also required.

For further information contact: Professor T Radesater, Department of Zoology, University of Stockholm, PO Box 6801, S-113 86 Stockholm, Sweden (Telephone 00946-8340860 ext 322).

Applications including curriculum vitae plus one copy of published, accepted papers, a short summary of the applicants research and teaching experience, names and addresses of three referees and any other papers the applicant wishes to submit should be addressed to The Board of Stockholm University, Fedrik Bloms Hus, University of Stockholm, Sweden, and received no later than March 1, 1982. Quote reference number 728/81.

(W492)A

## FACULTY POSITIONS IN PATHOLOGY

The Uniformed Services University School of Medicine invites applications for tenured or tenure track faculty appointments in the Department of Pathology at the levels of Professor, Associate Professor, or Associate Professor. Levels of appointment and salaries will be commensurate with training and experience. Candidates should have MD, PhD or related professional degrees, appropriate postdoctoral training or experience, teaching abilities, and demonstrated excellence in research.

Interested candidates should submit curriculum vitae, bibliography, and names of three professional referees by 30 November 1981 to Dr Robert Friedman, Chairman, Department of Pathology, c/o Personnel/Manpower Division, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20014. The Uniformed Services University is an equal opportunity/affirmative action employer. (NW061)A

### PLANT ECOLOGIST University of California Davis

Applications are invited for a tenuretrack faculty position at the assistant professor and assistant botanist levels, available 1 July 1982. The individual selected will be expected to develop a vigorous research program in plant population and community ecology, to teach undergraduate and graduate courses in botany and plant ecology, and develop a strong graduate training program. The appointment is split between the College of Letters and Science (.6) and the Agricultural Experiment Station (.4) and has a salary commensurate with the assistant professor range (11-month appointment) at the University of California. Send applications with curriculum vitae, statements of research and teaching interests (including long-term goals), transcripts, and request that 3 letters of recommendation be sent to: Dr. R. W. Pearcy, Chairperson of the Search Committee, Department of Botany, University of California, Davis, CA 95616. Applications must be postmarked by 1 January 1982. The University of California is an Affirmative Action/Equal Opport-unity Employer. (NW067)A unity Employer.

## BROWN UNIVERSITY

## POSTDOCTORAL RESEARCH ASSOCIATE

To work on on an experimental study of the infrared spectroscopy of van der Waals molecules of atmospheric significance. A recent Ph.D. in physical chemistry preferred with experimental experience in one or more of the following: molecular beams, IR spectroscopy, diode lasers, apparatus/computer interfacing.

Send résumé and names of three referees by 1 December to: Professor J.M. Calo, Division of Engineering, Box D, Brown University, Providence, Rhode Island 02912. An Equal Opportunity/Affirmative Action Employer. (NW058)A

## **ENTOMOLOGIST**

Assistant Professor, 12-month, tenure-track position for insect ecologist with background in medical/veterinary entomology to conduct research on biting flies affecting man and animals in New Jersey and to teach undergraduate and graduate courses in medical/veterinary entomology and insect ecology, or in his/her specific interest area. Position is budgeted as two-thirds research, one-third teaching.

Send application, including résumé, transcripts, and three letters of reference, to: Dr H.T. Streu, Department of Entomology and Economic Zoology, Rutgers — The State University of New Jersey, New Brunswick, N.J. 08903.

(NW059)A

## Molecular Genetics of Yeast

A yeast or microbial Geneticist is required to join a group involved in the genetic modification of brewing yeasts. The person appointed will work for the UK's largest brewing group in the Research Department, located in Burton-on-Trent, Staffordshire.

The successful applicant will contribute to the development of genetic systems for brewing yeasts, including recombinant DNA techniques and their application to achieve technical and commercial objectives. He/she will possess a Ph.D. in a relevant field of genetics or molecular biology and have made extensive use of genetic techniques during their research, possibly with some post-doctoral experience. Practical experience with protoplast fusion and/or cloning is desirable, but not essential.

Applicants should be innovative and possess the initiative to progress towards defined objectives. The person appointed will be encouraged to maintain and extend liaison with academic laboratories and to publish results where appropriate.

Salary will be commensurate with age and experience. The Company operates a generous life assurance and pension scheme and provides comprehensive recreational facilities.

Detailed applications, including a curriculum vitae and the names of two referees, should be sent as soon as possible to:-

Recruitment and Training Manager, Bass Brewing Limited, Burton-on-Trent, Staffordshire, DE14 1JZ.

## Bass Limited



(9883)A

### UNIVERSITY OF HEIDELBERG Germany

## **Graduate Research Assistant**

Applications are invited from graduates having or expecting to obtain a 1st or upper 2nd class degree.

The successful applicant will work on mechanisms of gene expression (structure-function relationship of procaryotic promoters, terminators, rib. binding sites).

Methods to be used will include: chemical and biochemical manipulation of DNA signal sequences, cloning in plasmids, DNA sequencing, quantitation of gene products in vivo and in vitro.

The salary will be between 1,800 and 2,000 DM per month.

Applications (including curriculum vitae and the names and addresses of two referees) should be sent to Prof. Dr Hermann Bujard, Institut für Molekulare Genetik, Universität Heidelberg, Im Neuenheimer Feld 230,6900Heidelberg, FRG. (W487)A

## THE DEPARTMENT OF PHYSIOLOGY & BIOPHYSICS is seeking candidates for two new FACULTY POSITIONS

Applicants must have a doctoral degree and several years of postdoctoral experience, and should be prepared to carry out independent research & to participate in the teaching activities of the department. We will consider applicants in any area of physiology or biophysics, but will give preference to people working in the area of cellular neurobiology, membrane physiology & theiregulation of metabolism.

We plan to appoint tenure-tract associate & assistant professors, but will also consider applicants for appointment as tenured asseciate and full professors.

Applicants should send their curriculum vitae and bibliography. Reprints of several recent papers a statement of current research interest, and the names of three references to: Dr Robert L Perlman, Head, Department of Physiology & Biophysics, University of Illinois College of Medicine, PO Box 6998 Chicago, IL 60680.

(NW973)A

## **PSYCHOPHYSICIST**

Unilever Research, Port Sunlight, wishes to recruit a Scientist experienced in recent developments in psychophysical theory and methodology

For this post we are looking for a Scientist having a First Degree in Psychology followed by either:

- (a) a Ph.D in Psychophysics or
- (b) a Ph.D in a relevant subject such as physical or biological science or experimental psychology,

plus in either case at least three years of post-doctoral research in psychophysics.

The Company operates a progressive salary and promotion policy, good superannuation and life cover benefits and where appropriate, will assist with removal expenses, legal fees, etc.

The Laboratory is conveniently situated for access to Liverpool, Chester and North Wales.

Please write for an Application Form Quoting Ref: PS 733 M to:

> **Personnel Officer Recruitment & Placement Unilever Research Port Sunlight Laboratory** Quarry Road East, Port Sunlight, Wirral Merseyside.



## **UNIVERSITY OF ZIMBABWE**

Applications are invited for the following posts

Academic

## LECTURESHIP/SENIOR LECTURESHIP IN THE **DEPARTMENT OF GEOLOGY** (available 1 April 1982)

Applicants must have a sound knowledge of metamorphic and structural geology. Minimum qualification is a PhD and practical field experience is essential.

Research Fellowships

## **FACULTY OF SCIENCE (2 posts)** (available 1 January 1982)

Applicants should have a PhD degree in one of the following disciplines: biochemistry, Botany, Computing Science, Chemistry, Geology, Mathematics, Physics or Zoology.

Salary Scales (approx. Stg. equivs.)

Academic Staff

Lecturer Grade II:

Lecturer Grade I:

 $\begin{array}{l} £\ 5,291\times380-£7,191+398-£9,181\\ £\ 9,604\times398-£11,196\\ £10,600\times398-£11,794\times408-£13,835 \end{array}$ Senior Lecturer:

Research Fellows

Junior Research Fellow: £4,013 × 263 - £4,802 Research Fellow Grade II: £5,064 × 344 - £6,440 £7,483 × 389 — £9,042 £8,516 × 389 — £11,243 Research Fellow Grade I: Senior Research Fellow:

Further particulars on the above posts, on conditions of service and on method of application should be obtained prior to submitting an application from Director, Appointments & Personnel, University of Zimbabwe, PO Box MP. 167, Mount Pleasant, Salisbury, Zimbabwe or from the Association of Commonwealth Universities (Apps), 36 Gordon Square, London WC1H 0PF.

Applications should be submitted by 30 November 1981 for Academic posts and 20 November 1981 for Research Fellow posts, or as soon as (9871)A possible thereafter.

### POSTDOCTORAL POSITION

available for Cell Biologist with strong background in biochemistry to investigate the structure and function of organelles involved in cell wall formation by plant cells and protoplasts. Some knowledge of plant tissue culture and/or electron microscopy is desirable. The position is available immediately for a one year term (renewable for a second year) at a salary of \$15,000.00 to \$18,000.00 depending on experience and qualifications. Some travel assistance is available.

Interested candidates should send curriculum vitae and 2 letters of recommendation to Dr L C Fowke, Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0WO.

(NW026)A

## **BIOTECHNOLOGY** RESEARCH

Applications are invited from

## **BIOCHEMISTS** or **MICROBIAL PHYSIOLOGISTS**

to join a group studying microbial biotransformations. Ideally, candidates will have some post-doctoral experience and will have worked on microbial biotransformations catalysed by oxygenases. The research is industrially funded.

Salary will depend on qualifications and experience and will be within the range £6,070 to £7,700 per annum. Initially, the appointment will be for a period of two years.

For an application form and further information contact: Personnel Section, Cranfield Institute of Technology, Cranfield, Bedford MK43 OAL. Tel Bedford (0234) 750111, ext 477. (9889)A

## UNIVERSITY OF WINNIPEG

DEPARTMENT OF BIOLOGY MICROBIOLOGY

Applications are invited for a

TENEURE STREAM POSITION AT THE RANK OF ASSISTANT, ASSOCIATE OR FULL **PROFESSOR** 

Candidates should have a PhD and relevant experience in teaching and research

Teaching duties will include a course in general microbiology plus other duties as required.

Salary dependent on qualifications and experience.

Applications together with the names of three references should be sent to: Dr R A Woods, Chairman, Department of Biology, University of Winnipeg, Winnipeg, Manitoba R3B 2E9.

Closing date: when position is filled. Starting date: not later than August 1st, 1982. Only Canadians or permanent residents need apply for this position. (NW068)A this position.

## UNIVERSITY OF THE WITWATERSRAND Johannesburg, South Africa DEPARTMENT OF CHEMISTRY POLYMER RESEARCH **PROGRAMME** SENIOR RESEARCH OFFICERS/RESEARCH **OFFICERS**

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin for appointment to the above posts. Work on challenging and unconventional aspects of organic and organometallic (including macromolecular) chemistry is being done. Applicants should have a liking for demanding (notably synthetic) experimentation, although no previous specialized experience is required. Appointees to pre-doctoral post may apply to work for a higher degree on a part-time basis. Only highly motivated applicants with good academic records will be considered. Appointments will be for one to three years in the first instance (with the possibility of renewal).

Intending applicants should obtain the information sheet relating to this post from the Secretary, South African Universities Office, Chichester House, 278 High Holborn, London WCIV 7HE. Tel: 01-405 5834, or from the Director: Personnel Office, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, 2001, with whom applications should be lodged not later than 30 November,

## UCLA SCHOOL OF **MEDICINE**

DEPARTMENT OF MICROBIOLOGY AND **IMMUNOLOGY** 

## **FACULTY POSITION**

Academic appointment for tenure track Assistant Professor with training in the area of macrophages as they relate to pathogenesis of diseases. Applicant must possess postdoctoral experience in the general fields of Biology, Microbiology and/or Immunology. Applicant must be able to develop an independent research program and interact with members of Department with emphasis in Microbiology, Immunology and Virology. Responsibilities include participation in the Departmental teaching of Medical, Dental and/or Graduate students, as well as development of a graduate course in the field of interest.

Applicants should submit curriculum vitae, list of publications, a brief description of current and future interests, and names of at least three referees. Deadline for receipt of applications: January 31, 1982. Send applications to: Ben Bonavida, Associate Professor or Jack Stevens Professor & Chairman, Dept of Microbiology and Immunology, UCLA School of Medicine, University of California at Los Angeles, Los Angeles, CA 90024. UCLA is an equal oportunity and affirmative (NW060)A action employer.

## UNIVERSITY OF WESTERN **AUSTRALIA** Perth **POSTDOCTORAL** RESEARCH ASSOCIATE IN GEOCHRONOLOGY

Applications are called for appointment to the above-mentioned position n the Department of Geology for a period of two years commencing early n 1982. The appointee will work with Ors M J Bickle, D I Groves and J R de Leater on an ARGC Funded project on the evolution of early Archaean crust of the Shaw Batholith, Pilbara Block, Western Australia.

The project is part of an on-going ield-based study of the structural hermal and chemical evolution of the early Archaean crustal segment exposed in the Shaw Batholith, a particularly well exposed area, showing a transitional character etween a gneiss terrain and a granitoid-greenstone terrain. The aim of the project is to date the tectonic volution of the area using Rb-Sr, Pb-Pb, U-Pb in zircon and Sm-Nd sechniques. The applicant should have expertise in solid source mass spectrosopy and clean laboratory chemistry Experience of U-Pb in zircon methods would be an advantage.

Salary range: \$A17,083 — \*A19,570 per annum. Some assistance will be provided towards relocation expenses.

Further information can be obained from either Associate Professor I Groves, Department of Geology, Jniversity of Western Australia, Nedands, Western Australia, 6009, or Dr

J Bickle, Department of Mineraland Petrology, University of Cambridge, Downing Place, Cam-wridge CB2 1TN.

Applications in duplicate stating ull personal particulars, qualifica-ions, experience and the names and ddresses of two referees should reach he Staffing Officer, University of Western Australia, Nedlands, Vestern Australia 6009, by 23 December 1981. (9878)A

## RESEARCH ASSOCIATE **AND** POSTDOCTORAL FELLOW

Positions immediately available to study biochemical and cell biological aspects of cholinergic excitation as part of an active research group devoted to this area. Previous experience in tissue culture, membrane biochemistry or in work with monoclonal antibodies would be advantageous.

Send curriculum vitae and the pames of two referees to Dr A Maelicke, Max-Planck-Institute, Rheinlanddamm 201, D-4600 Dortmund, West Germany. (W488)A

## X-RAY **SPECTROSCOPIST**

## for Geochemical Exploration Johannesburg - South Africa

In the Anglo American Research Laboratories a vacancy exists for an X-ray Spectroscopist/Geochemist preferably with experience in computer programming. Recent graduates will be favourably considered. The laboratories. situated in Johannesburg, function in a service, research and development capacity for the Anglo American Corporation. Although involved in a diversity of operations, the Corporation is principally a mining concern. Prospecting on an extensive scale is a vital function and X-ray fluorescence is one of the more important techniques employed for the determination of a large variety of elements in the prospection samples.

The most sophisticated equipment is used, including two multi-channel units with automatic sample changers. One of these units incorporates both wavelength and energy dispersive spectrometers, enabling automated simultaneous determination of many elements with high sample throughout. Computer programs are used for both on and off line data manipulation, tabulation, statistical evaluation, interpretation and contour mapping of analytical results. In certain aspects of the work the XRF laboratory is considered to be a world leader. Research and development of new methods and expansion of programs are on-going operations, for which well qualified personnel are required.

Employment benefits include a guaranteed 10% annual bonus, housing loan, furniture loan scheme, settling in and removal allowance and provision of temporary accommodation, free luncheons, pension and medical aid scheme and group life insurance.

Please write with full personal and career details, quoting reference SN 81/81, to: Ms. Sandy Cook, Anglo Charter International Services Ltd., (Appointments Division), 40 Holborn Viaduct, London, EC1P 1AJ

## ABOUT SOUTH AFRICA

Our climate lends itself to outdoor life and all sporting facilities are readily available. Our income tax rates are lower, VAT is only 4 per cent and the cost of living is lower than in the UK. Our schooling is of a high standard and we pride ourselves on the calibre of our medical facilities

South Africa has many attractions - we hope that you will want to share these with us.



## LONDON OFFICE

Jean Neville 4 Little Essex Street London WC2R 3LF Tel: 01 240 1101 Telex: 262024

## NEW YORK OFFICE

Cathy Moore 15 East 26 Street New York, NY 10010 Tel: (212) 689 5900

## TORONTO OFFICE Peter Drake Associates

32 Front Street West 201 Toronto, Ontario Tel: (416) 364 1623 M5J 1C5



## **University of Utrecht**

The Subfaculty of Physics and Astronomy of the Rijksuniversiteit Utrecht invites applications for the position of

## **Professor of Biophysics**

in the Biophysics Department. The candidates should have broad experience in the applications of physical techniques to structural studies of biological systems and will be expected to participate in the research activities of the department. The research programme includes optical spectroscopy, electron spin resonance, pulse radiolysis and dielectric studies of the dynamic structure of plant and model membranes. Duties also include teaching at undergraduate and graduate level within the Subfaculty. Candidates should be willing to serve on subfaculty boards and committees. Further details can be obtained from Professor Y.K. Levine, Biophysics Department, Fysisch Laboratorium, Rijksunversiteit Utrecht, P.O. Box 80.000, 3508 TA Utrecht, The Netherlands, tel. (030) 532363/631653.

Gross salary, depending on age and experience, ranges from Dfl.6352 to Dfl.9075 per month.

Applications, including a curriculum vitae, should be sent to the Chairman of the Selection Committee, Professor H.W. de Wijn, Fysisch Laboratorium, Rijksuniversiteit Utrecht, P.O. Box 80.000, 3508 TA Utrecht, The Netherlands, before 15 January 1982, under no. IL/2.013. Suggestions of suitable candidates will also be appreciated. Candidates should be willing to learn Dutch within two years.

(W489)A

## UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH WELSH PLANT BREEDING STATION

Aplications are invited for the post of

## SCIENTIFIC OFFICER (I) HIGHER SCIENTIFIC OFFICER (I)

in the Station's Grassland Agronomy Department. This is a new post created under the Welsh Hills and Uplands R&D programme. The successful candidate will join the Department's exiting Hills and Uplands team but will carry out most of the field work at Pwllpeiran Experimental Husbandry Farm.

Candidates should possess a First or upper Second Class honours degree in agricultural, biological or soil science with at least two years relevant post graduate experience for appointment as HSO. Preference will be given to candidates with a PhD and a Knowledge of hill farming conditions.

Pay Scales: (SO) £5,176 - £6,964 (HSO) £6,530 - £8,589. Starting Salary according to qualifications and experience. Noncontributory pension scheme but male employees aged 18 or over contribute 1 ½ % to Widows and Orphans Fund.

Further particulars and application forms are available from The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed. Closing Date for applications is 27 November 1981. (Quote reference 68). LA TROBE UNIVERSITY Melbourne, Australia SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF GENETICS AND HUMAN VARIATION

## LECTURER (Tenurable)

A position is available for early 1982 for a lecturer with research experience in molecular aspects of eukaryote gene structure and expression. Applicants should have a strong training in general genetics as some undergraduate teaching in prokaryote genetics will be required. The appointee will be expected to be actively involved in research, including the supervision of honours and postgraduate students.

The facilities of the Department include glasshouse, growth cabinets, constant temperature rooms, a Cl containment room, analytical and other centrifuges as well as routine items of potential assistance for a molecular geneticist.

Applicants for the previously advertised fixed-term appointment will be considered and need not reapply

Salary: \$A19,821 - \$A26,037.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications marked confidential and quoting reference number 150/32/22 close with the Staff Officer, La Trobe University, Bundoora, Victoria, Australia, 3083 by 11 December 1981. (9859)A

## THE INSTITUTE FOR ADVANCED STUDY

will have several openings for members in theoretical physics and astrophysics for the academic year 1982-83. The positions are at a post-doctoral or higher level and applicants will be selected on the basis of their ability to do research in the areas of elementary particles, mathematical physics, astro-physics, plasma physics, general relativity and statistical mechanics. Preference is given to candidates who have received their PhD within the last year or two.

Postdoctoral members frequently collaborate with each other, with faculty members at the Institute or Princeton University, and with researchers at other institutions.

Appointments ae usually for no more than two years and support is typically full salary for postdoctorals and half salary for more senior persons. Women and minorities are encouraged to apply.

For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW 1006)A

## FACULTY POSITION IN SPACE PHYSICS

The Department of Physics at Utah. State University is seeking candidates for a tenure track faculty position. Preference will be given to senior applicants who are actively working in space physics, particularly in the areas of stratospheric research, atmospheric chemistry of space plasma physics. The position will involve teaching the graduate or undergraduate level, guidance of graduate students and continuing research work within the center for Atmospheric and Space Sciences. Rank and salary will be commensurate with experience.

Please send résumé, a statement of research interests and the names of three professional references by December 31, 1981 to: Dr W J Raitt, Chairperson, Search Committee, Department of Physics, UMC-41, Utah State University, Logan, Utah 84322, USA, Utah State is an Affirmative Action/Equal Opportunity Employer. (NW050)A

ASSISTANT PROFESSOR, Surface Science. Rensselaer Polytechnic Institute is seeking outstanding applicants for this tenure-track position in physical chemistry. Minorities and women are particularly encouraged to apply. The appointee will be expected to develop a vigorous research program and to teach in the chemistry department at the undergraduate and graduate levels. Curriculum vitae, list of publications, and summary of research plans should be submitted, and arrangements made for three letters of recommendation to be sent, to James P. Ferris, Chairman, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12181. RPI is an Equal Opportunity/Affirmative Action Employer. (NW057)A

## NATIONAL COLLEGE OF AGRICULTURAL ENGINEERING (A School of Cranfield Institute of Technology)

## TEACHING ASSOCIATE POST

Applications are invited for a Teaching Associated post in Agricultural Science and Production to assist academic staff with their teaching and related work.

Candidates should have a good honours degree in a relevant discipline and desirably have experience of tropical and temperate agriculture. The post is tenable for two years in the first instance. Salary, within the range £5,285 — £7,700 per annum.

An application form and further particulars may be obtained from: The Head of Administration. National College of Agricultura Engineering, Silsoe, Bedford MK45 4DT. Telephone Silsoe, (0525) 60426 (9890)A

## PUBLIC HEALTH LABORATORY SERVICE BOARD

Central Public Health Laboratory — Colindale Applications are invited for

JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICERS (Grade B)

or

## STATE REGISTERED MEDICAL LABORATORY SCIENTIFIC OFFICER POSTS

Candidates applying at Junior B level should possess 'A' Levels in Chemistry and one other science subject or an appropriate Science Degree and be prepared to follow the relevant courses leading to State Registration. Training is given in all aspects of Microbiology and will include a period spent in a routine clinical microbiology laboratory outside Colindale.

Currently, vacancies exist in Divisions of Enteric Pathogens and Microbiological Reagents and Quality Control as well as within the Central Organisation.

Further information and application forms can be obtained from the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, Colindale, London NW9 5HT. Telephone 01-205 7041.

(9858)A

ASSISTANT, ASSOCIATE or Full Professor of Entomology to teach insect behaviour and conduct fundamental and/or applied research in insect behaviour with application to practical problems. PhD required. Submit curriculum vitae, transcripts, description of research experience and goals, summary of teaching experience, reprints of publications, dissertation summary, names of 3 referees to H K Kaya, Chair, Search Committee, Department of Entomology, University of California, Davis, CA 95616 by December 31, 1981. An Equal Opportunity/Affirmative Action Employer.

(NW056)A

## YALE UNIVERSITY DEPARTMENT OF GEOLOGY AND GEOPHYSICS

Applications are solicited for a faculty position in solid earth geophysics to begin in the academic year 1982-83. Areas of interest to the Department include seismology, exploration geophysics, mechanical and physical properties of rocks and minerals, geomagnetism, and tectonophysics.

Yale University is an equal opportunity/affirmative action employer and encourages women and members of minority groups to compete for this position.

Curriculum vitae, publications and the names of three or more referees should be sent by 31 December 1981 to Robert B Gordon, Chairman, Department of Geology and Geophysics, PO Box 6666, New Haven CT 06511. (NW1010)A

## HAMMERSMITH HOSPITAL

DEPARTMENT OF MEDICINE

## BASIC GRADE BIOCHEMIST

£5,988 — £8,396 pa, according to qualifications and experience. Required for Hormone Assay Laboratories in the Endocrine Unit.

The laboratories provide service radioimmunoassays for Hammersmith Hospital, the Regional Health Authority and the Supra-Regional Hormone Assay Service; and are engaged in research in most aspects of endocrinology, particularly pituitary disorders.

Science Degree required, and previous experience in this field is an advantage.

Application forms and further details available from the District Personnel Department, Hammersmith Hospital, Du Cane Rd, London W12. Tel: 01-743 2030 ext 49.

N.B. This is a readvertisement of an MLSO post: previous applicants need not apply. (9881)A

## GRAVITATIONAL WAVE DETECTION AND EXPERIMENTAL GRAVITY — LSU

A postdoctoral position is available in the gravitional radiation group at Louisiana State University. We have a cryogenic tuned bar detector which is sensitive to strains of  $10^{-17}$  at  $4.2 \, \text{K}$ . We are developing a Josephson junction parametric amplifier as part of the detection scheme. We are also investigating the applicability of various quantum-non-demolition techniques. Other investigations related to experimental gravitation may be undertaken.

Inquiries from women and members of minorities are encouraged. Contact W O Hamilton, Department of Physics and Astronomy, LSU, Baton Rouge, La. 70808. LSU is an equal opportunity employer.

(NW049)A

## UNIVERSITY OF EDINBURGH

DEPARTMENT OF MOLECULAR BIOLOGY Applications are invited for a

## POST-DOCTORAL RESEARCH

position to investigate the molecular mechanism of conjugal DNA transfer by bacterial plasmids. Experience in molecular genetics and/or in vitro DNA manipulation is desirable. The appointment is available from 1 January 1982, and will be for 2 years. Salary on Research Range 1A at a point commensurate with age and experience

Applicants should send a CV, reference and letter describing their research interests to Dr N S Willetts, Department of Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh EH9 3JR. Please quote Reference 5064.

л. (9880)А



## Universität Hamburg

Im Fachbereich Biologie ist ab sofort im Anthropologischen Institut zu besetezen:

Eine Stelle, Kennziffer 844/3

## Professor(in) der Bes.Gr. C3 für Anthropologie

Aufgabengebiet: Vertretung des Faches in Forschung und Lehre. Bewerber (innen) sollen in der Forschung experimentelle Arbeiten auf dem Gebiet der Humanbiologie durchfürhren und in der Lage sein, im Unterricht vor allem die Sero- und/oder zytogenetik des Menschen zu vertreten. Es werden anerkannte wissenschaftliche Leistungen im anthropologischhumangenetischen Bereich, Überblick über verschiedene Teilbereiche der Humanbiologie und Lehrerfahrung auf humanbiologischem Gebiet erwartet.

Lehrverpflichtung: 8 Semesterwochenstunden.

Einstellungsvoraussetzungen: Gem. § 15 Hamburgisches Hochschulgesetz.

Bewerbungen mit tabellarischem Lebenslauf sowie vorerst nur Schriften — und Lehrverzeichnis werden unter Angabe der Kennziffer.

bis zum 28.12.1981.

erbeten an den Präsidenten der Universität Hamburg, Verwaltung/Personalreferat, Moorweidenstraße 18, 2000 Hamburg 13. (W495)A

NEUROPHYSIOLOGY / Respiratory Physiology: Research Associate Position, available immediately, for studies of neural and chemical control of ventilation. Doctorate required and postdoctoral experience preferred. Salary commensurate with experience. Curriculum vitae and names of three referees should be sent to: Walter M St John, PhD, Department of Physiology, Dartmouth Medical School, Hanover, NH 03755, USA. (Dartmouth College is an equal opportunity, affirmative action (male/female) employer.)

## POSTDOCTORAL RESEARCH ASSOCIATE

position available for research on the plasma factors regulating platelet functions in the field of thrombosis and hemostasis. Experience in biochemistry and/or immunology preferred. Salary 24K per year.

Send curriculum vitae, statement of research background, and three names for reference to: Eric Lian, MD (D26), PO Box 016760, Division of Hematelogy, University of Miami School of Medicine, Miami, Fla. 33101. An Equal Opportunity/Affirmative Action Employer.

(NW054)A

## ROLLS-ROYCE FELLOWSHIP AT WOLFSON COLLEGE, CAMBRIDGE

\*\*\*\*\*\*\*\*\*

A vacancy exists for a person with proven research ability in high speed aerodynamics to work and manage projects sponsored by Rolls-Royce at the Whittle Laboratory in the University's Engineering Department. The post is established for three yeras in the first instance and remuneration is on the University Lecturer scale. The successful applicant will be nominated for election as a Research Fellow at Wolfson College.

Further details and an application form may be obtained from the President, Wolfson College, Cambridge CB3. 9BB. Closing date for applications: 15 January 1981.

(9882)A

## YOU'VE GOT A **BRIGHT IDEA**

## FOR NEW BIOLOGICAL OR CHEMICAL COMPOUNDS

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We are not a pharmaceutical firm but a Swiss-based, independent financial group which is prepared to let you participate in a capital venture for the exploitation of your project.

Our object: the development of new biological or chemical products with previous pharmalogical screening up to the stage of clinical tolerance and efficacy required to permit licensing negortiations.

To obtain all the information required for initial selection, please write in English, French or German to:

DEBIOPHARM S.A. Petit-Chêne 38 - 1001 LAUSANNE (Switzerland).

(W368)A

## **WANT TO LIVE** IN THE U.S.A.?

American agency provides help. For free information write

the U.K. address. The Transatlantic Agency, (N),

33 Great James Street, London WC1. **Enclose SAE please** 

(9891)A

## university college of

Senior Research Assistant Applications are invited for the acancy of Postdoctoral Senior Research Assistant in the Department of Biochemistry. The successful applicant will work with Professor E. G. Brown and Dr. R.P. Newton on an SRCsponsored project concerning the isolated and complex-bound cyclic nucleotide phosphodiesterases of plant tissues. Preference will be given to applicants with experience in the extraction and purification of enzymes and/or the isolation of plant organelles.

The commencing salary is on a scale up to £6,880 per annum plus USS/USDPS benefits, and the appointment is for one year in the irst instance, renewable for a urther year.

Further particulars and application forms may be obtained from the Personnel Office, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to which office they should be returned by Friday, December 11, 1981.

## RESEARCH ASSOCIATE (Postdoctoral)

DEPARTMENT OF **PHARMACOLOGY** 

This position available for studies on the control of insulin release. Emphasis on calcium handling at the subcellular level and the role of calcium in exocytosis. Both physio-logical and biochemical techniques will be used.

Applications should be sent to Dr Geoffrey W G Sharp, Chairman, Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853. Cornell University is an Affirmative Action/ Equal Opportunity Employer.
(NW064)A

## HIGH ALTITUDE OBSERVATORY

Visitor Appointments at the High Altitude Observatory are available for new and established PhD's for up to one year periods to carry out research in solar physics, terrestrial physics, and related subjects. Applicants should provide a curriculum vitae including education, work experience, publications, the names of three scientists familiar with their work, and a statement of their research plans. Applications must be received by 15 January 1982, and they should be sent to: Visitor Committee, High Altitude Observatory, National Center for Atmospheric Research (NCAR), PO Box 3000, Boulder, Colorado 80307, NCAR is an Equal Opportunity/Affirmative Action Employer. (NW931)A

### THE DISTILLERS **COMPANY LIMITED ASSISTANT SCIENTIST**

The Food Group of the Distillers Company Limited require an Assistant Scientist for work in the Spirit Yeast Fermentation Laboratory of their Yeast Research Department, Menstrie, Clackmannanshire.

Applicants should have a HNC qualification in chemistry, preferably with 2 or 3 years laboratory experience in the use of fermentation or biochemical techniques and including the handling of gas liquid chromatography equipment. Applications from candidates possessing qualifications other than HNC will not be considered further. The person appointed would be directly responsible to the Microbial Biochemist in charge and would assist him in research projects as well as in routine laboratory work with eventual responsibility for day to-day laboratory operations

An application form can be obtained

by writing to: The Yeast Research Manager, The Distillers Company Limited, Food Group,

Research Department, MENSTRIE,

Clackmannanshire FK11 7ES. Closing date for applications is November 20th, 1981. (9816) A

## **LECTURES**

UNIVERSITY College London (Gower St, WC1E 6BT). The Smith, Kline & French Lecture in Allied Health Sciences by Professor K. Murray, 'Opportunities for Genetic Engineering in Biomedical Science', Tues. 17 November at 5.30 in the Botany Theatre. Admission Free. Without Ticket. (9860)K

## **AWARDS**

### **POSTDOCTORAL AWARDS IN OCEAN** SCIENCE AND **ENGINEERING**

Woods Hole Oceanographic Institution invites applications for 1-year postdoctoral scholar awards from new and recent doctorates in fields of biology, chemistry, engineering, geology, geophysics, mathematics, meteorology, and physics, as well as oceanography. Recipients of awards are selected on a competitive basis, with primary emphasis placed on research promise.

Fellowship stipend is \$20,000. Appointees are eligible for group health insurance and a modest research budget. Recipients are encouraged to pursue their own research interests independently or in association with resident staff.

Completed applications must be received by 1 February 1982 for 1982–1983 awards. Awards will be announced in March. Write for application forms to: Dean of Graduate Studies, P.O. Box N. Woods Hole Oceanographic Insti tution, Woods Hole, Massachusetts 02543.

Equal Opportunity/Affirmative Action Institution (NW053)N

### **ASSISTANTSHIPS**

## THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF VIROLOGY

### Applications are invited for a RESEARCH ASSISTANTSHIP

funded by the MRC, and tenable for three years from 1 January 1982. The appointee will work on the immunogenicity of vaccines prepared from recombinant influenza viruses.

The work involves virological, immunological and biochemical techniques, including electron microscopic studies. A good honours degree in biological/biochemical subject required.

Initial salary of £5,285 a year (Range IB for Research and Analogous Staff), and the successful applicant will register for a PhD

Applicants should send their curriculum vitae, together with the names and addresses of two academic referees to Dr R Jennings, Department of Virology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX. (Tel. 0742 26484 ext 2019), by 27 November 1981. Quote ref: R645/G. (9884)P

## CONFERENCES and **COURSES**

## SCIENTIFIC **GLASSBLOWING**

Day and evening classes are held in the Science Department of Hounslow Borough College, St. John's Road, Isleworth, Middx. Tel: 01-568 0244. (9856)C

We would like to call the following announcement to the attention of your readers:

THE ERNEST WITEBSKY CENTER FOR IMMUNOLOGY State University of New York at Buffalo announces the

## EIGHTH INTERNATIONAL CONVOCATION ON IMMUNOLOGY

to be held June 14-17, 1982 at the Buffalo Marriott Inn,

Amherst, New York.

The theme of the program, "Regulation of the Immune Response' include presentation of papers by distinguished scientists internationally recognized as experts on the topics: Systematic Immuno-regulatory Mechanisms; Mucosal Immunoregulatory Mechanisms; Immune Regulation to Exogenous Antigens; and Implications in Clinical Disease States.

Further information may be obtained by contacting Dr James F Mohn, Director, The Ernest Witebsky Centre for Immunology, Room 210 Sherman Hall, State University of New York at Buffalo, Buffalo, New York 14214 USA, (telephone AC 716-831-2848).

(NW045)C



## HEAT SHOCK INDUCTION OF PROTEINS May 5-May 8, 1982

Organized by:

Michael Ashburner University of Cambridge

Milton J. Schlesinger Washington University

**Alfred Tissières** University of Geneva The conference will focus on such subjects as:

Heat Shock Genes & Their Organization.

Nucleosome Structure & Function in Heat Shock. Transcription & Translation of Heat Shock mRNAs.

Heat Shock Proteins: Structure, Localization in the Cell, Possible Functions,

Conservation of Structure among Species.

Biological Effects of Heat Shock: Protection, Role in Homeostasis.

Relation to Cell Transformation. Hyperthermia in Chemotherapy.

## IN VITRO MUTAGENESIS MEETING May 12-May 16, 1982

Organized by:

Robert Schleif Brandeis University

**David Shortle** 

State University of New York,

Stony Brook

**R. Bruce Wallace**City of Hope Hospital

The projected agenda will cover such topics as:

Directed Synthesis of Mutant Sequences.

Synthesis Using Base Analogues.

Site-directed Mutagenesis. Deletions.

DNA Repair In Vivo and Chemical Modification In Vitro.

Putting Mutagenized DNA Where You Want It.

Applications & Accomplishments.

## RNA PROCESSING May 19-May 23, 1982

Organized by:

John J. Dunn Brookhaven National Laboratory

Michael B. Mathews

Cold Spring Harbor Laboratory

Joan A. Steitz Yale University Topics to be covered will include:

Large and Small Ribonucleoprotein Particles.

Small RNAs.

RNA:RNA & RNA:Protein Interactions. tRNA, mRNA, and rRNA Processing.

mRNA Splicing.

To obtain further information and registration material for the above meetings and/or the complete summer meetings schedule, please contact:

Meetings Coordinator, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, New York 11724. (516) 367-8345

## **FELLOWSHIPS**

## E M B O

## **EUROPEAN MOLECULAR BIOLOGY ORGANIZATION** SHORT TERM FELLOWSHIPS in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of the application.

Applications for exchanges between laboratories within any one country cannot be considered.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, 69
Heidelberg 1, Postfach 1022.40, F.R.G. (W490)E

### **UNIVERSITY OF** BIRMINGHAM

**DEPARTMENT OF BIOCHEMISTRY** 

## RESEARCH FELLOWSHIP

Applications are invited for a Research Fellowship for three years to work with Dr N J Kuhn on an ARC sponsored project to establish and biochemically study mammary epithelial monolayers on floating collagen gels. Applicants should have suitable postgraduate experience, preferably including cell culture.

Starting salary within the range £6,070 — £6,880 pa (plus superannuation) depending on age, qualifications and experience.

Applications (2 copies) including a curriculum vitae and the names of three academic referees, should be sent by 30 November 1981, or as soon as possible thereafter to Jane Nelson, Senate Registry, University of Bir-mingham, PO Box 363, Birmingham, B15 2TT, from whom further particulars may be obtained. (9868)E

### THE WEIZMANN **INSTITUTE OF SCIENCE** Rehovot, Israel

Offers Postdoctoral Fellowships in the life sciences (biology, bio-chemistry, biophysics), chemistry (physical, organic, geological and biological), mathematical sciences, physics and science teaching. The fellowship provides a 12-month

stipend (which is adjusted to the costof-living index), a relocation allowance and one-way air-fare. Fellowships which are renewed for a second year provide round-trip airfare.

Application forms and additional in-formation may be obtained from the Feinberg Graduate School, of the Weizmann Institute of Science Rehovot 76-100, Israel.

The applications should reach the above address not later than May 15, 1982, for fellowships starting in September 1982 and not later than November 15, 1982, for fellowships starting in April 1983. (W496)E

### IMPERIAL CANCER RESEARCH FUND

**DEVELOPMENTAL GENETICS** LABORATORY (MILL HILL)

## **POSTDOCTORAL** RESEARCH FELLOWSHIP

Applications are invited for a three year postdoctoral appointment, starting September 1982, to work on the molecular genetics of pattern for-mation in *Drosophila*. The project involves the study of genes affecting the establishment of early embryonic pattern. The work makes use of recombinant DNA technology, genetic analysis and embryology, and experience of one of these techniques would be useful although not essential.

Salary range £7,700 to £9,750 plus Outer London Weighting (rate under review) with entry according to qualifications and length of post-doctoral experience.

For further information contact Dr D Ish-Horowicz at ICRF, Burtonhole Lane, Mill Hill, London NW7. Applications supported by full type-script CV, including date PhD ob-tained, list of publications and names and adresses of two referees, should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 by 31st December 1981 quoting reference 7/82. (9854)E 7/82.

## **STUDENTSHIPS**

## HANNAH RESEARCH INSTITUTE

University of Glasgow Applications are invited for a PhD STUDENTSHIP

tenable at the Biochemistry Department and funded by the UKDA Nutritional Consultative Panel, to work on the dietary regulation of hepatic and intestinal cholesterol metabolism.

Applicants must hold or expect to obtain a First or Second Class (Upper) degree in Biochemistry or closely related discipline and should send their cv and names of 2 academic referees to: The Secretary, Hannah Research Institute, Ayr KA6 5HL, (9875)F Scotland.

## THE ROYAL SOCIETY **BROWNE RESEARCH FUND**

**MAURICE HILL** RESEARCH FUND MARSHALL AND **ORR BEQUEST** 

## **GRANTS AND RESEARCH FELLOWSHIPS 1982**

The Council of the Royal Society invites applications from qualified research workers for personal grants:

(a) from the Browne Research Fund, for research in marine biology at a marine biological laboratory or elsewhere. Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end.

(b) from the Maurice Hill Research Fund (established in memory of the late Dr M N Hill, FRS) for research in the general fields of physical and chemical oceanography, marine geophysics and geology, either at sea or in laboratories and institutes having adequate facilities.

(c) from the Marshall and Orr Bequest for the support of marine biological research.

Grants may be used for periods up to one year, and are made on the understanding that university or other posts held by applicants will mean-while be kept open for them. Recipients are permitted to use grants while receiving salaries from regular appointments.

Applications are also invited for two one-year junior research fellow ships supported from the Maurice Hill Research Fund and the Marshall and Orr Bequest for applicants under 26 on 1 April 1982; these can be taken up any time between April and October 1982 and will be on the Society's Category D salary scale (currently £7,018  $\times$  £406 to £9.048 per annum with superannuation and national insurance).

In 1982 the total amounts available to be shared among all successful applicants will be about £9,500 from the Browne Research Fund, £15,500 from the Maurice Hill Research Fund and £17,000 from the Marshall and Orr Bequest; individual grants will not ordinarily be more than £1,500.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by 15 December 1981; applicants must state if they wish to apply for personal grants or for one of the junior research fellowships described above. (9862)E

## THE ROYAL SOCIETY JOHN MURRAY TRAVELLING STUDENTSHIP IN OCEANOGRAPHY AND **LIMNOLOGY 1982**

Applications are invited for the above Studentship, open to British graduates under the age of 35, for the encouragement of travel and work in oceanography or limnology. This award, which may be held for periods from three months to one year, will in 1982 be up to £2,000, adjusted according to qualifications and place and duration of research.

Applications are expecially invited from workers in oceanography and limnology, as well as in zoology, botany, geology, mathematics and physics, and it will be permitted to hold the award while receiving a salary from a regular appointment.

Applications receivable by 15 December 1981 should be made on forms obtainable from The Executive Secretary (BHM/UMAM), The Royal Society, 6 Carlton House Terrace, London SW1 5AG (9863)E

## **GRANTS**

## THE WELLCOME TRUST WELLCOME PROJECT DEVELOPMENT GRANTS FOR VETERINARY RESEARCH

The Wellcome Trustees announce their intention of making available in 1982, up to £150,000 for one or more Major Grants for the support of Veterinary Research to be carried out wholly, or in part, in a British University. The Grants, which may be held for up to five years, are intended to enable the expansion of an existing research programme along new and promising lines, or to promote the development of a separate project which could benefit from the expertise and resources currently devoted to research in a Department. The Trustees wish to maintain a degree of flexibility in the operation of these grants which may provide for research assistance and expenses, and essential items of equipment; the Grants are not intended to provide the personal remuneration of applicants.

The closing date for applications, which must be submitted on the appropriate form, is 1st March 1982.

Intending applicants are invited to address informal preliminary enquiries to Dr K B Sinclair, The Wellcome Trust, 1 Park Square West, London NW1 4LJ. (Telephone: 01-4864902). (9886)H

## **ANNOUNCEMENTS**

## 'SCIENCE AT THE CROSSROADS''

a meeting to commemorate fifty years of the British Radical Science movement.

Speakers

Margot Heinemann, Gary Wersky, Hilary and Steven Rose, Bob Young.

Friday, 20th November, 7.00pm Imperial College, Chemistry Theatre B Imperial Institute Road, SW7 Details: BSSRS, 9 Poland St, W1 (9853)G

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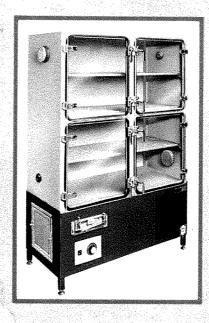
## INDEPENDENT ENVIRONMENTAL CABINET FOR ANIMAL STORAGE REF. E 110

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It provides renewal of atmosphere and, at the same time, maintains a positive or negative pressure of clean air, by means of HEPA filter, in the storage space as required for the animals stored.

This unit can be fitted with up to 16 medium-size cages (approximately 200 mice). An additional module can be installed above or beside the base unit in order to double the storage capacity.

It is also possible to foresee an heating system for the fresh air entering the cabinet, using electrical resistance heating, interlocked with an electonic control system (sensitivity  $\pm$  0,5 °C).



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Elle permet d'isoler les animaux en atmosphère contrôlée indépendante de l'environnement, sans répandre leurs od dans l'ambiance du local.

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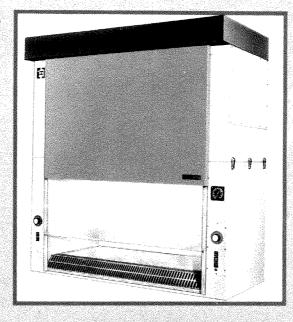
Cette enceinte peut recevoir jusqu'à 16 cages moyennes (200 souris environ). Un module supplémentaire peut êtri disposé au-dessus ou à côté de l'unité de base afin de de la capacité de stockage.

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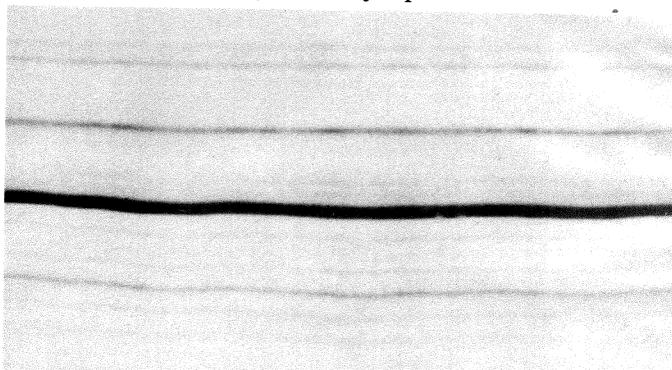


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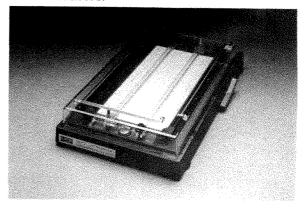
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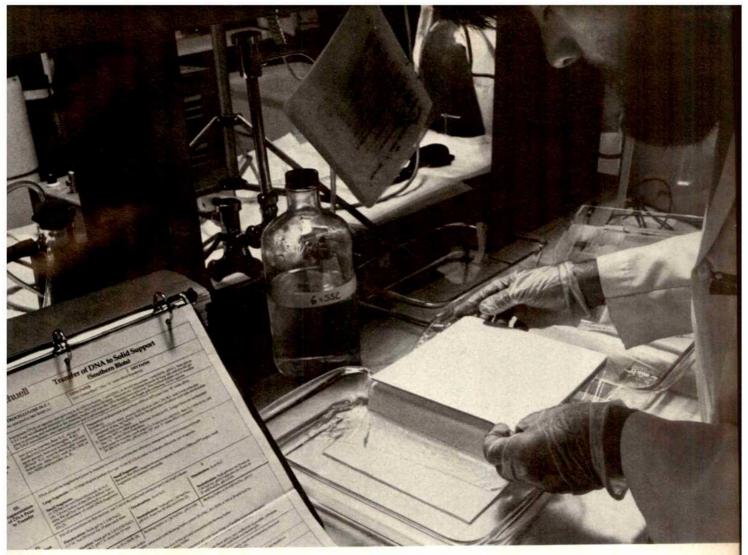
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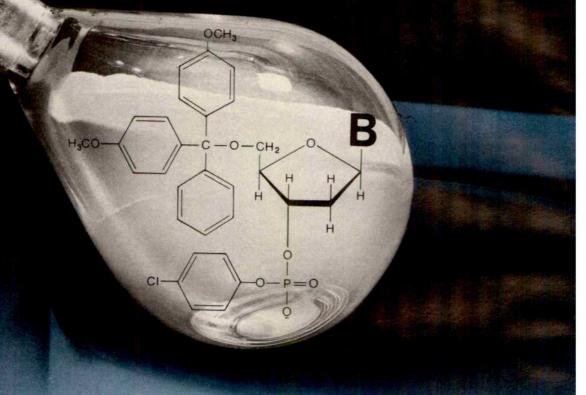
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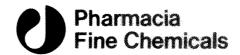
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dTTP, [methyl, 1', 2,'-3H]-

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DNA Polymerase I

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Deoxynucleoside Triphosphate

Mixture

Nick Translation Buffer Nick Translation Stop Buffer

Deionized Water

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5'-triphosphate)

Terminal Deoxynucleotidyl

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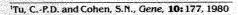
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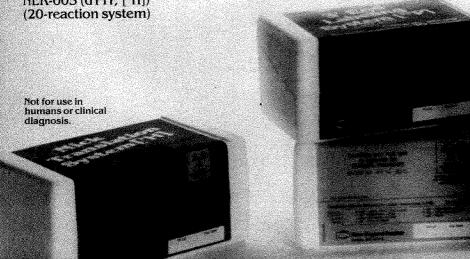
pBR322 (control to check system **Deionized Water** 

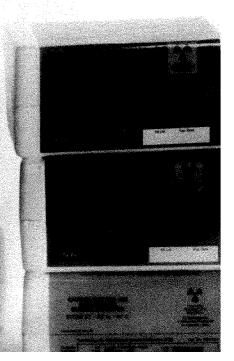
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#### W NA Sequencing

s system follows the Maxambert procedure as published in thods in Enzymology and ludes the entire text of the arti
2 It is suitable for sequencing A labeled with our 3' or 5' End beling Systems and includes all igents necessary for the four se specific cleavage reactions. Ifficient material is provided for at st fifty of each reaction.

e following components are :luded: Imethyl Sulfate ydrazine iperidine iperidine Formate erric Chloride odium Hydroxide iaxam-Gilbert Procedure Manual

ch new lot of components is sted before shipment in a seence analysis of a DNA fragment. ie purity of each component is onitored using chromatographic alysis and documented for clusion with the system.

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xam, A.M. and Gilbert, W., Methods in symology, 65 (1980)



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<sup>3</sup>Manley, J.L., Fire, A., Cano, A., Sharp, P.A., and Gefter, M.L., PNAS (U.S.A.), **77:** 3855 (1980)

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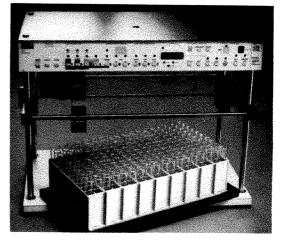
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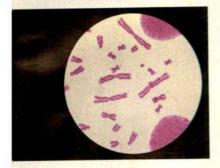
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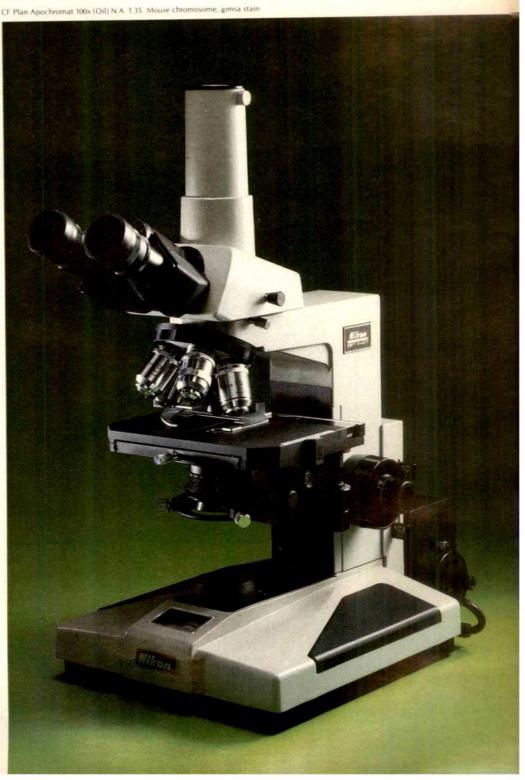
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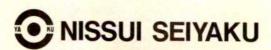
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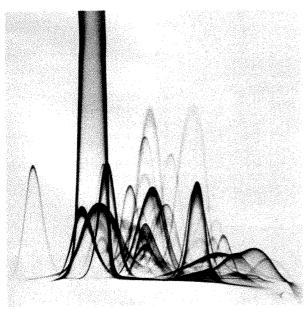
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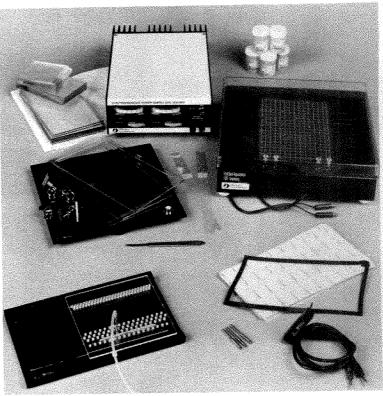
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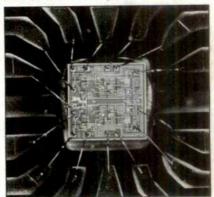
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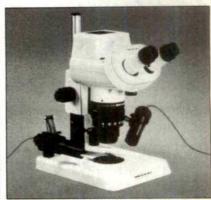
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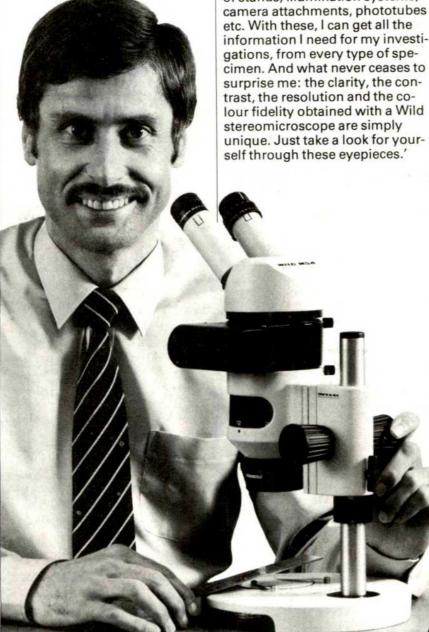
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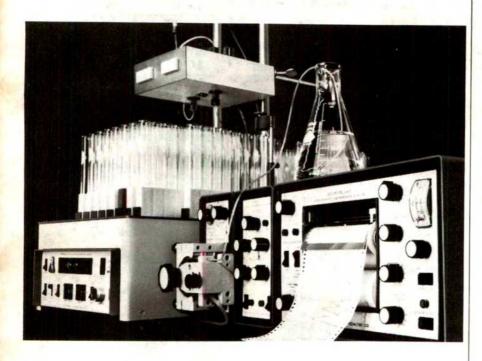
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#### 19 November 1981

#### More muddle about nuclear weapons

Does the United States know what it is saying on nuclear strategy? Does it appreciate the consequences in Europe? Will it properly represent European interests at the arms control talks beginning 30 November?

The government of the United States lacks a way with words. So much is clear from the declarations of President Reagan and his Secretaries of State and of Defense in the past few weeks about the likely course of nuclear war in Europe. All three members of the Administration have let slip statements on this complicated issue which are dangerously over-simple. Have these high officials of state never learned to qualify simple declarative statements of policy with the conditional clauses that are usually necessary? Whatever the explanation, their gaffes have thrown a cloud over the negotiations due to begin in Geneva at the end of the month between the Soviet Union and the United States on European nuclear weapons. People in Europe, to say the least, are wondering whether officials so unsubtle in their use of language can be trusted fairly to represent the European case at these crucial talks.

The dispute two weeks ago between Mr Alexander Haig and Mr Caspar Weinberger about NATO's plans for demonstrating a nuclear weapon in some future crisis says more about personal relationships within the Administration than about the nuclear defence of Europe. On the Wednesday, Mr Haig was telling the Senate Foreign Relations Committee that NATO's contingency plans include one to "demonstrate to the other side that they are exceeding the limits of toleration". The following day, Mr Weinberger told the Senate Armed Services Committee that there is no such plan - nor "should" there be. Later, the White House announced that both secretaries were telling the truth. So, plainly, they are. What Washington now calls a "demonstrational" nuclear weapon must obviously be an option for NATO to consider if a conventional conflict should break out in Europe. On the face of things, such a device would be preferable to the use of nuclear weapons against targets on the ground. But it is virtually impossible to tell in advance when such a demonstration might be effective. What the Secretaries of State and Defense were talking about was what NATO calls the doctrine of "flexible response". They should have said so.

President Reagan's own slip of the tongue is more serious. In answer to a question from a group of newspaper editors three weeks ago, he said it is conceivable that some future nuclear war might be confined to Europe. Many Soviet strategists share this belief, even though Mr Leonid Brezhnev consistently denies it with gloomy accounts of how any nuclear war would visit destruction on the whole world. Mr Brezhnev is probably nearer the mark than Mr Reagan, for if by evil chance a single nuclear weapon is used in anger, there is no obvious stopping place on the ladder of escalation. Yet the NATO doctrine of flexible response supposes that a conventional incursion across the North German plain would have to be countered, after a few days, by "tactical" nuclear weapons. The hope is that this response would bring the incursion to a halt. If that happened, Mr Reagan would be right.

Yet this calculation is a gamble, and one with long odds. The strategists agree that the conflict would more probably escalate and that the United States would itself be drawn in. Precisely that probability is the foundation for NATO's strategic assumption that nuclear weapons are a peace-keeping device, a deterrent. Moreover, the argument works in both directions. The decision in 1979 that American nuclear weapons in Europe should be modernized by means of Pershing II and cruise missiles was intended by the then United States Administration to strengthen

the linkage between Western Europe and the United States. It is simply wrong of Mr Reagan to fail to point that out when speculating about the possibility that a nuclear war might be confined to Europe. The most serious damage done by his and his colleagues' remarks, however, is that Europeans' willingness to accept the theory of deterrence, by its nature bizarre, has been further undermined. Washington's willingness to talk about nuclear warfare as if it were just an elaboration of conventional warfare suggests that it does not understand what its strategy is for.

What does this imply for the talks beginning on 30 November in Geneva? At first sight, the prospect is not bright. Past experience, however, has shown that there is nothing more educative of the superficial understanding of politicians than negotiations about arms control. Six months from now, President Reagan and his colleagues will be wiser men, perhaps even regretting their delay in sitting down to talk. Moreover, it now appears that the United States has moved a long way to accommodate the anxieties of European governments about nuclear weapons. Last weekend, the Administration let it be known that its opening bid at Geneva will be an offer to dispense with Pershing II and cruise missiles (due to appear in Europe in 1983) if only the Soviet Union will remove its SS20 missiles (together with the older SS4 and SS5 missiles). The Soviet Union will predictably reply that the American offer says nothing about the French and British nuclear weapons. Sooner or later, one side or the other will acknowledge that aircraft cannot be entirely ignored. And the United States will have to decide what to do about Salt II.

Even so, the months ahead are potentially fruitful for the cause of arms control. Indeed, next year will also bring a special assembly of the United Nations on arms control, which could be valuable if states with only marginal interest in the subject can be prevented from making speeches about instant and comprehensive disarmament. There may also be a conference on arms control in Europe within the framework of the Helsinki agreements. It would make sense that the issue of a comprehensive ban on testing nuclear weapons should be given another airing, ideally as part of a Salt III, but the chances of that are slim - too much is at stake for the superpowers readily to agree. The potential beneficiaries of any progress in these directions are several. Many states, in Eastern as well as Western Europe, would welcome the civility and the greater political flexibility that further measures of arms control would bring. Even the superpowers, however, should benefit - President Reagan should be glad if part of his prospective budget deficit melts away,

There are, however, several potential snags. Just as negotiations on arms control are not merely better than no negotiations but are also in themselves a source of reasonableness, arms control negotiations that break down are a source of danger. The United States government has only itself to blame if its self-contradictions of the past few weeks have raised doubts about its seriousness, and its stomach for the tedium that lies ahead. More seriously, the proposal that the opening bid in the Geneva negotiations should abolish all potentially strategic nuclear missiles in Europe is likely, before many months have passed, to boomerang. The American missiles were, after all, intended as a guarantor of American involvement in European defence; will not their absence seem to many a proof of American detachment?

Nobody should be surprised if, some months from now, present anxieties about nuclear weapons based in Europe are outmatched by fears that, without them, one half of Europe or the other will be defenceless.

Finally, there is the problem — strictly, the red herring — of Mr Brezhnev's proposal for an international committee of distinguished scientists to consider the consequences of nuclear war and to tell the world what they are. In the past few months, the Soviet Union has introduced resolutions to this effect in the general assemblies of the World Health Organization and the UN Environmental Programme. By all accounts, a similar proposal will soon be put to the general assembly of the United Nations. In reality, however, there is no shortage of documents spelling out the horrors of nuclear conflict. Most of them agree that the consequences would be appalling. What is needed is not another chilling document but a more constructive attempt to show how nuclear war can realistically be avoided. While the scientific community has much to contribute to such an investigation, it has no special competence to say what is strategically and politically possible. But that, of course, is why the two superpowers are sending delegations to Geneva.

#### **Equity in research**

British research councils are saying that universities must also invest in research. But can they? And fairly?

The Medical Research Council has helpfully made public the letter in which it has told British universities that they need not apply for research grants on behalf of department which are inadequately equipped (see page 201). By this means, universities will at least know where they stand. And it is only right that a substantial grant-making agency such as the council, which is in principle well placed to compare the utility of money spent in university departments and in its own establishments, should do what it can to ensure that grants are not wasted because universities cannot give their recipients adequate support. One problem, of which the council must be well aware, is that in the process of saying no to applicants whose universities cannot support them properly, it may be denying good people a run for their money. For who can be sure that the academic departments which universities decide must be cut back will never include among their staffs potentially creative people? By doing what it must - concentrating support in the departments that universities themselves decide to back generously — the council (like other British research councils) is in danger of backing mediocre horses. This is another way of saying what has been clear all along — that the dual-support system (by which universities pay for the overhead cost of academic research, and research councils for the extras) has long since broken down. The question now is what should be done to put things right. To remark that a committee under Sir Alec Merrison has been brooding on the question for the past two years is not a sufficient answer.

The plain truth is that it will be intolerable (but also a dangerous waste of talent) if research councils such as the Medical Research Council think it prudent not to back particular people because the universities concerned have chosen to be mean to the departments in which those people work. The simple solution is obvious but probably unattainable — let the British research councils pay the full cost of the research projects that they back, transferring the cost of the overheads they would then incur from the budget of the University Grants Committee: the universities would kick up too much of a fuss. Another would be to consider grant applications from all university departments, and to use research council money to help ensure that successful applicants are able to migrate to places where research could be carried out effectively. The device now used in the Netherlands (see page 202) by which universities are subsidized under the two separate headings of teaching and research would be even less welcome to British universities — but, on that account, might be an efficient spur to change. Whatever stratagem is used, there is a crying need that something should change. For to be unable to make research grants to people likely to use them well is not merely inequitable to those concerned but unfair to the rest of us.

#### Come back, Columbia

The shuttle looks like a success. Might it be a better one if NASA had time to think?

The second test-flight of the shuttle spacecraft was in no sense the near-calamity it has been represented to have been. That one out of three fuel cells should have functioned inefficiently, but that the spacecraft should nevertheless have performed well for more than two days, can just as well be taken as a proof of the good sense of its designers and of the flexibility of the system they have devised. It is not as if the shuttle were simply another kind of aircraft, whose development would no doubt have followed a more conventional course, with a succession of test-flights gradually extending its performance. Instead, it has gone almost in one jump from the drawing-board to full flight. In the steady enlargement of the regimen accessible to manoeuvrable passenger-carrying vehicles, the shuttle is a landmark of an innovation. Two years late though it may be, the US National Aeronautics and Space Administration deserves full credit for making it fly at all.

Two groups of problems nevertheless persist. First, the cost of the remaining development threatens to be an insupportably large fraction of the total space budget, with the result that NASA cannot sustain its modest interest in the less spectacular parts of its research programme even though it has been shielded from the full rigours of the 12 per cent budget cut decreed last month. It



cannot be that this is what the White House intends. And even though the potential military importance of the shuttle has led some to suggest that the Pentagon might shoulder some of the responsibility for it, its importance as an economical means of launching satellites of great commercial importance is, rather, an argument (if one were needed) that budget-balancing by means of across-the-board cuts is no way to conduct government business. But there are also problems with the shuttle itself, last week's success apart. Now, not later, is the time to be sure that solutions adopted ten years ago to technical problems such as the heating of the spacecraft in the upper atmosphere are still the best.

The important need, now, it to get the shuttle right. The United States space administration is understandably anxious not to delay much more. The customers are restive, and the political dangers for an agency that fails to deliver could be serious. But, to many people's surprise, space travel in the modest mould represented by last week's Columbia has come to stay. It will be economically important and, in the long run, a cheerful influence on people's spirits. The space agency will no doubt say that all the technical problems — the engines, the heat shield, the turn-round time and the next rocket stage — are continually being looked at. It would be best if this re-examination could be comprehensive, and public. For then the pressures from would-be customers, at the Pentagon and elsewhere, would be diminished. As would the risk of losing great benefits for want of a little forethought.

## US plans help for nuclear industry

# Civil power to link with weapons?

Washington

The Reagan Administration has offered the US nuclear industry all of the moral — and some of the economic — support that it wants. But even this may not be enough to restore the industry to health according to the parting words of the Nuclear Safety Oversight Commission (NSOC), an independent advisory body set up by President Carter following the Three Mile Island accident in March 1979.

Three main options face the Administration if it wants to save the civil nuclear industry, according to Governor Bruce Babbit of Arizona, chairman of NSOC. He presented President Reagan with this opinion when transmitting the commission's final report last month. Babbit warned, however, that each of the options would require a high political price.

The first option would be to regionalize the nuclear industry and draw it into the public power grids, a form of seminationalization already adopted, for example, by the Tennessee Valley Authority. The second would be to bail out the industry directly through government subsidies, although allowing it to remain largely in private hands.

The third and perhaps most controversial option would be to re-establish links between the commerical uses of nuclear power and the military demands for nuclear weapons, using the expansion of the latter to sustain the former. Some critics argue that the Administration's plan to use plutonium extracted from commercial wastes to provide fuel for weapons is already a step in this direction.

This drastic set of choices reflects the serious problems facing the industry. The most popular scapegoats have been the tough environmental regulations and stringent licence review procedures; but no less important has been the reduction in the rate of growth in power demand from 7 to 3 per cent a year over the past decade, and continued public concern about safety (highlighted by the design mistakes found in the Diablo Canyon reactor under construction in California).

The Administration has already taken several steps to assist the nuclear industry. In an attempt to encourage nuclear exports, for example, it is offering countries such as Mexico cut-price enriched uranium, and scientific collaboration in areas such as waste disposal and reactor safety.

Other measures are being taken to speed

up the licensing of new plants. In a policy statement issued last month, Mr Reagan listed the goal of reducing the time needed to license and operate a new nuclear reactor from an average of 12 to 7 years as one of five steps intended to revitalize the nuclear industry (*Nature* 15 October, p.505).

Congress is likely to be sympathetic. Two weeks ago the House of Representatives passed a bill that would allow utility companies to start initial low-level operations of a new nuclear plant even before all local complaints had been fully heard. A similar measure, now before the Senate, is expected to pass with little difficulty.

The economic and safety problems facing the industry are unlikely to be resolved as simply. In the past few months several local utilities have decided to abandon plans for new reactors. In several cases, such as the Pilgrim II plant which was to have been built by Boston Edison Co. on the Massachusetts coast, construction delays and the need to incorporate new safety requirements have increased initial estimates of construction costs by about a factor of ten.

Many industry supporters feel that, in view of growing consumer opposition to the rapidly escalating electricity bills to



Waiting at Diablo Canyon

finance such construction — in some cases increasing by 500 per cent within a few years — the only way for the industry to remain viable is through a massive infusion of federal funds. But this possibility is already coming under fire from both sides

#### Irish veterinarians in dog-house

Brussels

A report by the European Commission on the eradication of cattle diseases has levelled an accusing finger at the Irish veterinary profession. Since 1965 about £90 million has been spent by the state on eradicating bovine tuberculosis in Ireland but, says the Commission, "there has been no change in the bovine tuberculosis situation in Ireland".

"It should not be concluded that this lack of progress is due to any lack of finance (£11 million was spent in 1980) but rather to the fundamental fact that twothirds of the reactors are being missed at annual round testing" continues the report. The Commission does not accuse anyone of cheating. It does, however, draw a comparison with the results obtained during the early 1970s by about 50 temporary veterinary inspectors employed by the Department of Agriculture to carry out tuberculin tests on a random sample of herds that were normally assigned to local veterinary surgeons for testing. The inspectors tested about 18 per cent of the national herd over several years and found approximately three times as many infected animals and herds as had the vets.

It would, of course, be to the farmers' advantage to avoid having their animals identified as being infected as this would involve high costs. Apart from the restrictions on the movement and sales of the animals and the need for proper handling

and isolation facilities, the amount of compensation paid for slaughtered beasts does not match full market value, in contrast with the situation in the United Kingdom.

Faulty testing schemes may be the answer and the European Commission, which is proposing to continue with its accelerated programme to eradicate brucellosis, tuberculosis and leukosis in the EEC, is threatening to cut off disease eradication funds to Ireland unless testing procedures are improved.

The EEC's contribution to the shared costs of accelerating the eradication of these diseases is some 130 million European Currency Units (about £65 million) over the past three years. The Commission confidently reports that by 1983 at the latest, all EEC heads will be under control as far as brucellosis and tuberculosis are concerned. By the end of the current programme, it is estimated that 1.5 million animals will have been destroyed.

Enzootic bovine leukosis will almost certainly be eradicated in Denmark in the near future and the disease is well under control in Germany. France and the United Kingdom have not availed themselves of EEC funds to eradicate leukosis. The Commission also studiously avoids contributing to the debate in the United Kingdom on the badgers and tuberculosis issue except to say that the disease has been practically eradicated there.

Jasper Becker

of the political spectrum.

The Administration has already agreed to support a federal contribution of \$123 million towards the almost \$1,000 million which it is estimated will be needed to clean up the Three Mile Island power plant. Private utilities have agreed to contribute a similar amount through the Edison Electric Institute, and the rest of the money comes from insurance cover and from the states of Pennsylvania and New Jersey.

The extra costs of new plants is not merely a result of licensing delays. As safety questions come under scrutiny, embarrassing facts are beginning to emerge. At the Diablo Canyon plant in California, for example, the wrong blueprint had been used to calculate potential stresses arising from an earthquake. And almost all of the operating nuclear plants across the country are likely to miss the deadline imposed by the Nuclear Regulatory Commission for replacing between 15 and 40 per cent of their electrical equipment which had previously been thought safe, but which was shown to be liable to failure under exposure to steam and radiation that might occur during an accident.

Such design errors have played into the hands of anti-nuclear protesters who claim that nuclear technology must remain under strict control and supervision. The industry claims that it is being strangled by these controls; but, especially at a local level the courts have tended to back up the critics.

These developments appear to foreclose all but the final NSOC option — that of increasing links with the military sector, perhaps through the recreation of the Atomic Energy Commission which shared responsibility for the military and civilian uses of nuclear fission until the early 1970s. Department of Energy officials, for example, are already claiming that their initial proposals to extract plutonium from commercial wastes through laser isotope separation (*Nature* 30 July, p.401) could partially solve the storage problem.

Here again, however, the political problems are likely to be enormous. Critics argue that allowing the military greater leverage over the civilian programme could threaten attempts to control nuclear technology through more democratic means, and that it would conflict with efforts to limit the proliferation of nuclear weapons in developing nations by trying to divorce the civilian and nuclear aspects of nuclear energy.

Nobody in Washington pretends that finding the solution will be easy, President Reagan has asked Energy Secretary James Edwards and the director of the Office of Science and Technology Policy, Dr George (Jay) Keyworth, to consult industry, the utilities and universities, and they have been given almost a year to prepare a report on "obstacles that stand in the way of increased used of nuclear energy and the steps needed to overcome them".

David Dickson

#### Human growth hormone

#### **Shortage persists**

British supplies of human growth hormone are in danger. Over-optimism about the availability of the hormone from genetically engineered bacteria combined with a failure to appreciate that even mortuary workers are human has left the United Kingdom's National Health Service with supplies which are inadequate for the optimal treatment of the 800 British children with growth hormone deficiency.

Until biotechnology raised the prospect of an alternative, the only source of human growth hormone was the pituitary glands of cadavers. Three years ago at least 50,000 pituitary glands were collected from mortuaries in hospitals and processed in Cambridge. Another 20,000 pituitaries were collected from public mortuaries for processing in London. The combined operation, under the auspices of the

#### Two heads for one

The Centre National de la Recherche Scientifique of France now has its complement of two heads, a directorgeneral and a president, just two weeks after the previous incumbents resigned on a matter of principle. First — as reported last week — the mathematician Jean-Jacques Payan has been appointed director-general; and now M. Claude Frejacques, present director of the Délégation Générale à la Recherche Scientifique et Technique (DGRST) has been appointed president.

The appointments may be interim ones, as the Minister of State for Science and Technology, M. Jean-Pierre Chevènement, is said to prefer a single director for CNRS, rather than the dual headship established by the previous administration. But there was no time to change the constitution before the national colloquium, due in January, where major policy issues will be thrashed out in public, and CNRS—as the major supporter of basic research in France—has to have a clear voice by then.

Nevertheless, the appointment of Frejacques, a career civil servant rather than a scientist, has its rationale. DGRST was effectively administration of the previous - and less powerful - science minister, Pierre Aigrain, and Chevènement has begun to set up almost a rival administration in his new ministry. DGRST may in the end be disbanded in all but name, with its parts becoming wings of the research ministry. Chevenement already has a chef du cabinet, so some role had to be found for Frejacques. CNRS seemed to suit - now leaving the minister free to shuffle DGRST as he Robert Walgate wishes.

Medical Research Council, would have produced more than enough growth hormone for British needs so that up to half of the pituitaries from hospital mortuaries were stockpiled. Because of a drastic fall in the collection of pituitaries from hospitals, the stockpile is now depleted. Faced with a huge rise in cost of the hormone, the Department of Health and Social Security (DHSS) has now ordered a reduction in therapeutic dosage during 1982.

Trouble began when DHSS took over the collection and processing of pituitaries. Most of the hospital pituitaries were to be processed in the department's new Centre for Applied Microbiological Research at Porton Down. Material from public mortuaries, on the other hand, was to be handled by the Swedish company Kabi Vitrum, chosen because it has the European rights to manufacture and market human growth hormone from bacteria genetically-engineered by the Californian company Genentech. Bacterial growth hormone was to have been provided by Kabi Vitrum to DHSS at a preferential price as soon as British clinical trials, due to start in January 1981, had been successfully completed.

The first snag with these plans was that DHSS decided to use the changeover as an opportunity to consolidate into the wages of mortuary workers at hospitals, the small sum that had previously been paid to them for each pituitary collected. As many a manager might have told DHSS, consolidation can be a recipe for diminished productivity. The number collected from public mortuaries has fallen to about 13,000 a year despite the reinstatement of the special payments, but was never more than 20,000. The combined annual collection of pituitaries has therefore fallen by 60 per cent and now provides less than half the amount of hormone needed to treat British children.

The shortfall has been exacerbated by a delay in the production of bacterial human growth hormone. The first batch of Genentech's hormone to be given to humans had unacceptable side effects (fever and the lysis of blood monocytes) and full clinical trials had to be postponed. The side effects were almost certainly due to the presence of bacterial toxins in the hormone preparation, and both Genentech and Kabi Vitrum have now developed a more complex purification process. Genentech claims that its cleaner preparation has cleared toxicity tests and says that it is already six weeks into a clinical trial on children. Kabi Vitrum is slightly behind, having just completed a toxicity trial in Sweden.

Some, however, doubt whether the bacterially-derived human growth hormone will pass through its clinical trials successfully. The scepticism is based on the fact that the bacterial hormone is not quite identical with the authentic human hormone. Clever though Genentech's genetic engineers are, they have not been

able to devise a way for their bacteria to produce growth hormone without an extra methionine at one end of the molecule. This amino acid, say some hormone biochemists such as Dr Philip Lowry of St Bartholomew's Hospital, is a prime target for recognition by antibodies. He therefore predicts that when used in long-term therapy, bacterially-derived human growth hormone will provoke an antibody response that will preclude its clinical use.

It is too soon to know whether Dr Lowry's predictions are right. If not, Kabi Vitrum bacterial growth hormone should be available to plug the gap in Britain's traditional supplies before dosage has to be too seriously reduced. The reduction already planned is considered not to be disastrous; one expert reckons that at worst the children so treated would end up no more than three centimetres short of their maximal height.

Because there is a world shortage of the hormone there is little hope that DHSS will find alternative supplies. And even if it could, the cost would probably be prohibitive. One estimate is that 3 years ago, the cost was £1.25 per 5-unit ampoule whereas an equivalent ampoule purchased by DHSS from Kabi Vitrum now costs £15.

Peter Newmark

#### UK medical research

#### To those that hath...

British university departments asking the Medical Research Council (MRC) for research grants will now need an assurance of financial support from their universities. MRC's chief concern is that universities, in adjusting to their own reduced budgets, may starve individual departments of funds, with the result that its research grants are "rendered ineffective".

In a letter to universities this month, the council reaffirms its belief in the dual support system, under which British university departments winning grants from research councils are supposed to be maintained as "well-found laboratories" out of the general subvention from the University Grants Committee. It acknowledges, however, that there may be temporary difficulties, as when universities decide to freeze vacant posts.

The move is MRC's attempt to force the universities' hands. Like the Science and Engineering Research Council, it is concerned that the quality of university research will be irreparably damaged if universities spread their dwindling resources too thinly. Hence it will support the objective of the University Grants Committee (UGC) that universities should concentrate their own resources on good departments considered worthy of support. Applicants for new grants from institutions not favoured by UGC may be in for a raw deal.

The MRC, nevertheless, offers some help to universities. Researchers who lose their jobs, for example, will be eligible for small personal grants, which, while not paying their salaries, will help them complete projects already started. Universities are also invited to nominate exceptionally promising researchers who may be eligible for help. MRC is willing to expand its senior fellowship scheme which provides support for up to ten years for promising young researchers unable to find tenured posts. Demand for the scheme, which has been running for four years, is already heavier this year than in the past. MRC is also willing to increase the number of awards it makes under existing schemes which free academics from teaching and administrative tasks to allow them to spend a few years on full-time research.

The release of the MRC letter to the universities last week coincided with publication of its annual report for the year ending March 1981 (HMSO £4.00). According to the report, the council has resolved its long-standing dispute over the terms of employment of researchers on short-term contracts. The council has agreed that 70-80 per cent of its posts will carry tenure, compared with 67 per cent previously. Short-term appointments will now be almost exclusively for three years and open competition will be invited for tenured positions. The new scheme will not cost the council more, according to Dr James Gowans, Secretary of MRC, chiefly because it does away with appointments of intermediate term.

During 1980-81, the council spent nearly £93 million, about £15 million of which was transferred to its budget from the Department of Health after the collapse of the Rothschild customer-contractor principle. Under the principle, first introduced in 1974, money was transferred from the council's annual budget to government departments for spending on research commissioned through the council. The new policy, however, has made little difference to the council's work, according to Dr Gowans, because the health department chose to commission long-term research which the council will continue to support. In the deal struck with the health department, the council has agreed to increase support for health service research to £2 million by 1985-86.

The year to March 1981 was not easy the council had to supplement its government grant with £500,000 of its own money and was not able to provide all the support for top quality research for which it was asked. But Dr. Gowans's chief concern is for the future. In particular, he fears that the government may renege on its earlier promise to maintain the real value of the science vote to be announced in December. The implications of a real budget cut for MRC, which in any one year has more than 90 per cent of the its budget tied up in on-going commitments, could be far-reaching, involving a reduction in the amount of research it supports in universities. Judy Redfearn European Science Foundation

#### Signs of solidity

Strasbourg

The European Science Foundation (ESF) — Europe's fledgling international academy — appears to have come of age. Last week the foundation, representing 47 research councils in 18 countries, made its first direct approach to governments with a letter to research ministers requesting them to take up the idea of a "European Synchrotron Radiation Source", a £30 million third-generation source of X-rays for Europe.

The new source is considered necessary by most European X-ray users if Europe is to keep abreast of American competence in the field. But the foundation's request is significant not only for its content but as an example of the foundation's new confidence in the practical role it can play in Europe.

The foundation has previously been cautious of its role vis à vis its member research councils, but there seems to be such agreement among the various councils over the synchrotron source, such a need to cooperate financially in the present recession and such growing confidence in the foundation's offices that the members are willing to let the corporate ESF approach go ahead.

Professor Hubert Curien, the French president of the foundation, makes it clear in his letter to the ministers that ESF—as a non-governmental organization—cannot handled political questions such as the site for the source or the national contributions to its cost, but requests governments to set up a committee of representatives to do just that. The governmental committee would then work at arm's length from the foundation, "seeded", as it were, by the foundation's earlier enthusiasm, hard work and the now-detailed specification of the X-ray machine.

How governments will respond is yet to be seen, but already several countries and organizations have made unofficial offers of sites, the most detailed of which has come from Italy (for Trieste). The ESF committee has also made a careful study of the possible use of a tunnel at the European Centre for Nuclear Physics (CERN) near Geneva, which now holds the intersecting storage rings, a ten-year-old device likely to be closed within a few years to save money for the large electron-positron collider (LEP). If the new X-ray source were built at CERN it would, however, come outside the CERN budget.

According to the foundation's optimum timetable, the intergovernmental committee would meet early in 1982; governments would take a decision in principle in early 1983; and the source would be in operation by 1988-89.

The foundation also agreed last week—at its annual assembly in Strasbourg—on a serious study of what may be its next big project—a "geotraverse" of Europe. This

would entail a detailed geological survey down to the mantle, using every available technique, of a band 2 to 20 kilometres wide stretching from the North Cape in Norway to North Africa, across the many major ancient geological boundaries of Europe.

The exercise would be a continental parallel to the International Deep Sea Drilling project and would cost around £3 million over seven years, 2-3 years of which would be used to gather data. The Swiss national research council will pay for a pilot study.

On a smaller scale, the foundation has decided to set up a new fellowship scheme this for toxicologists - similar to its existing European Training Programme in Brain and Behaviour Research. Each year £70,000 will be made available by council in nine countries (Denmark, Finland, Ireland Italy, The Netherlands, Norway, Sweden and the United Kingdom) for short-term and long-term fellowships in toxicology. The object is to stimulate research on the toxicology of environmental chemicals and to help increase the number of experts in Europe who could advise governments and industry. The first fellowships will be advertised next spring to be available in autumn 1982. **Robert Walgate** 

#### **EEC Research Council**

#### **Broader future**

Brussels

EEC's ten research ministers have finally approved the first stage of a biomolecular engineering programme and the 4-year programme on microelectronics (worth \$40 million). This agreement, reached at a meeting on 9 November, reflects the future attitude of the Community to research and development.

The European Commissioner for industry and now research, Etienne Davignon, sees research and development as being one of the prime vehicles by which Europe's flagging industrial competitivity compared with the United States and Japan can be revived. That his ideas are being taken seriously by member states is demonstrated by the council's decision to go ahead with biomolecular engineering and microelectronics programmes.

Only the first stage of the 4-year programme (indirect action) on molecular engineering has been agreed. The original six comprehensive projects proposed by the commission still stand, but the whole programme will now be focused on agriculture and on safety and environmental questions. So for two years and with about \$8 million to spend on 50 per cent support, the commission will fund research on, say, the synthesis of vaccines and pesticides of importance to European agriculture; on the biotransformation of agricultural surpluses and wastes; and on plant molecular genetics and gene transfer. The safety work - accounting for 20 per cent of the grant — will cover the detection of contaminants in industrial microbial strains and the extension of risk assessment procedures.

After two years, the programme will be re-assessed and if successful continued — probably with a further injection of cash. The commission hopes to call for tenders around the end of this year, and the programme will start in earnest on 1 April 1982.

The commission's 4-year action programme in microelectronic technology is the second arm of the strategy to stimulate European research into telematics and informatics. A programme has been under way since September 1979 on data processing and a third programme on telecommunications is expected to be proposed before the end of 1981. The agreed budget is for \$40 million, \$12 million less than was originally asked for.

This programme is also important because member countries have agreed to coordinate their activities and keep each other informed of new developments to ensure that a microchip production industry is soon established in Europe.

Figures from a report being prepared on the competitivity of European industry illustrate the struggle facing Europe. Jobs created in Europe between 1970 and 1980 numbered 2 million compared with 19 million in the United States and 5 million in Japan. Japan spends globally half as much money on research as EEC, but Japanese researchers register four times as many patents.

In the field of microprocessors, EEC is calculated to have spent \$470 million developing chips compared with Japanese expenditure of \$240 million. But Japan and the United States each supply 40 per cent of the world microprocessor market, while European production accounts for less than 10 per cent.

The commission's desire to coordinate research efforts carried out at national levels would involve holding regular twice-yearly meetings to plan and exchange information and analyse national spending. By discussing programmes at the early stages, overlapping and duplication could be avoided and lead to an efficient dissemination of research results both among the member states and between universities and industry. Using Euronet as an industrial data base and the planned INSIS integrated numerical network, the gap between research and industrial application would be narrowed.

For the Community's joint research centres, Davignon foresees the scope of the research being widened — a move that might involve the opening of the centres to agricultural research for the African, Caribbean and Pacific countries linked to EEC by the Lomé convention. The concept of promoting "centres of excellence" is also being discussed.

Agriculture research will also be boosted. Only 1.1 per cent of EEC's

research budget is devoted to this field despite the vital role the Common Agricultural Policy plays in EEC affairs.

Although the commission seems to be backing the argument that increased research and development is a means of solving current economic problems, a belief supported by European industrialists, ministers were non-committal on Davignon's request to double between now and 1986 the amount of money from the Community budget actually devoted to research and development.

Jasper Buker

#### Netherlands universities

#### Misery ahead

Ending several weeks of uncertainy the Netherlands government announced on Monday the latest forward plan for the universities. Briefly, the Ministry of Education and Science is looking for a 2 per cent cut in university salary budgets in the vears 1984 and 1985, together with a 3 per cent cut in other expenditure. Although the percentage reductions of the university budget (expected to save a total of 75 million Dutch guilders (£16.5 million) a year) are not at first sight large, coming as they do after several years in each of which university budgets have been reduced by 3 per cent, the consequences could be serious.

In the two years ahead, the ministry has also decreed that there should be a freeze on academic vacancies. During that period, the ministry also hopes that there will be a rationalization of the structure of university departments, with resources concentrated in the stronger departments. The Academic Council, which advises the ministry, has already begun to put individual departments in order of merit. It is possible that if the universities concerned do not take the initiative in reorganizing themselves, the minister will provide an incentive by adjusting the grants they are offered in the years ahead, either up or down.

One curious features of this week's proposals is that the government expects the universities collectively to pay their bills less promptly. The result may be that the drain on the government's cash resources is reduced by up to 40 million guilders in 1982. The reactions of the universities' creditors are not yet known.

On the face of things, there will be no immediate need of redundancies among academic staffs, although the ministry has set up a central register of vacancies. Even during the two years ahead, universities will be free to apply for a dispensation to fill vacant posts considered essential to their academic or research programmes. There is, however, a possibility that some universities will prefer to reduce their staffs than to stomach for a further two years the acute shortage of disposable income from which they have been suffering.

The next step will be for the parliament

in the Hague to approve the two university budgets — for teaching and for research — now proposed. Further support for research projects is provided by the Netherlands research council (ZWO) whose financial importance in university affairs has been increasing in recent years.

However, ZWO will have to do without a science minister — a post established only eight years ago. The previous minister, Anthonius van Trier, was without portfolio, but he badgered his ministerial colleagues into giving him control of substantial parts of their budgets. The new government has removed this irritation, the support of science reverting to the new minister of education, Josephus van Kemenade. And as Kemenade's major political interest is in establishing comprehensive education for Dutch schoolchildren, scientists in the Netherlands are worried.

#### Agricultural research

#### **Changes mooted**

Washington

"Reach out and touch somebody", the slogan being used in the United States to promote the use of long-distance telephone calls, has also become a newly-prominent policy theme for the thirteen agricultural research institutions which constitute the Consultative Group for International Agricultural Research (CGIAR).

The group is an informal network organized under the auspices of the World Bank through which developed countries, multilateral agencies and private foundations channel their support for research at the institutions into developing countries' agricultural needs.

At their annual meeting in Washington last week, both donors and research administrators endorsed current efforts by the institutions to increase linkages with outside research workers in two directions. One is to strengthen collaborative research projects with universities and other institutions in developed countries, in order to make the best use of present scientific knowledge. The second direction is to improve links with national agricultural research programmes in the developing countries.

It was the tenth annual meeting of the group, which has grown from four to thirteen members since it was founded in 1971. Since then, the total amount of funds channelled through the CGIAR system has risen from \$20 million to \$135 million.

In the past few years, however, as the rate of inflation has crept upwards, the growth of the institutes in real terms has begun to slacken off. Last year, for the first time, several institutes had to trim their programmes when it was realized that with various industrialized countries — and private institutions — cutting back on their aid programmes, voluntary contributions would not meet the targets.

#### Sowing more seeds

Mexico City

One of the biggest and best known of the research centres funded through the Consultative Group on International Agricultural Research (CGIAR) is the International Maize and Wheat Improvement Center (CIMMYT, after its Spanish initials), whose headquarters nestle in foothills on the edge of a high, fertile plain thirty miles north of Mexico City.

Based on a programme initially set up by the Mexican government and the Rockefeller Foundation in the early 1940s, CIMMYT was formally established as an international centre in 1966, and was one of the four founding institutes when CGIAR was created in 1971. Its current budget makes the largest financially of the 13 centres which now constitute the group.

In terms of its initial mission — the use of scientific breeding techniques to increase the yield of wheat and maize crops — CIMMYT has been spectacularly successful. It is known internationally as the home of the Green Revolution, due to the high-yielding varieties of dwarf wheat for which its most famous scientist, Dr Norman Borlaug, was awarded the Nobel prize. Among its more recent success stories is Bangladesh, which has increased its wheat yield from 114,000 tons in 1975 to 1.2 million tons in the current year.

But times are changing at CIMMYT. Although the mainstream research continues along conventional lines, CIMMYT is increasing its efforts in "farm systems research", addressing questions such as crop management and agricultural economics. Greater emphasis is being placed on the development and dissemination of improved research procedures to national research programmes, as well as on the support of indigenous training programmes.

One of the more controversial issues the centre faces is the growing demand on both sides of the Atlantic for greater patent protection for plant breeders. Dr Borlaug, acting director of CIMMYT's wheat programme, has criticized the new legislative initiatives, arguing that they could make developing countries more vulnerable to exploitation by unscrupulous outside companies. But others at CIMMYT seem prepared to accept, if reluctantly that tighter patent rights could accelerate the dissemination of new agricultural technologies.

The changing economic dynamics of food production are producing their own tensions within CIMMYT. Until now the centre has been distributing its germplasm with no charge and virtually on request. But after recent incidents, the rules are being tightened up.

Some recent scientific developments are also being watched warily. For example, there is scepticism about the size of the potential contribution of recombinant DNA technology to the direct improvement of crop yields, and CIMMYT has no molecular biologists on its staff. "We think that Wall Street is being widely optimistic", says CIMMYT's director general, Dr Robert D. Havener, referring to the heavy investor demand for shares in the new genetic companies, and quoting Dr Borlaug's view that "it will be 50 years before there is any significant impact on complex plants coming out of genetic engineering".

At the same time, however, CIMMYT is strengthening its links with research scientists in developed countries so that they can exploit any major breakthrough. Meanwhile Dr Havener's principal concern is to ensure that political and economic pressures do not upset the arrangement under which centres such as CIMMYT operate with minimum outside interference and the maximum amount of flexibility.

David Dickson

This year the situation looks as if it will be even tighter. Pledges for donations made at last week's meetings totalled \$155 million, an increase of about 15 per cent over the currrent year. However, according to Mr Warren C. Baum, the present high inflation rates plus fluctuations in the exchange rates covering the currencies in which donations are made mean that real growth will be small.

The combination of financial stringency and structural changes in the international research environment stimulating various shifts in strategy. One has been to increase the emphasis on basic research either at the institutions themselves or through links with the international scientific community.

Complementary to this will be the efforts

to assist national research programmes. In the past, tensions have arisen when donors faced difficult choices over whether to allocate funds to a particular country's research effort or to the international institutions. At a meeting held earlier this year in Nairobi, Kenya, for example, representatives from several African countries expressed strongly their view that CGIAR as a group should be doing more to assist indigenous efforts in their region, a message which is now being acted upon.

Keen to maintain the minimum of bureaucracy, last week's meeting reacted equivocally to a suggestion from the review committee that a new committee be established to coordinate budget request and allocations, and the issue has been deferred.

David Dickson

#### **Animal committees**

SIR — Your article "Protection for laboratory animals?" (Nature 17 September, p.173) presents a rational and realistic appraisal of the present dilemma concerning the ethical use of animals in experimentation being faced by the scientific community, the animal welfare community and the legislator.

It was of significant interest to the Canadian Council on Animal Care (CCAC) that the article concluded with the suggestion of building into a new system of regulation the establishment of "a committee at every important centre at which animals are used that would be empowered to sanction (or not to sanction) proposed uses of laboratory animals". The CCAC, established as a committee of the Association of Universities and Colleges of Canada in 1968 "to work for the improvement in the care and use of animals on a Canada-wide basis", has had, as its cornerstone, the development of local committees on animal care, at every institution in the country using animals in research. It was, and is, the intention that this committee should act as the "conscience" of the institution in ensuring ethical animal use is practised.

We recognize that we are dealing with human beings whose abilities and motivation vary. Therefore, some committees are more effective than others. However, it is our objective to continue to monitor the activities of these committees, in order to ensure that the weaker ones are made strrong, and the stronger ones continue to be strengthened.

It is our opinion, and one based on experience, that a local committee comprising conscientious and strongly-motivated members can do more than legislation to ensure the ethical treatment of animals in research.

H.C. ROWSELL

Canadian Council on Animal Care, Ottawa, Ontario, Canada

#### On chemical war

SIR - As noted by David Dickson (Nature 1 October, p.327) and Philip Campbell (Nature 22 October, p.598) the last few months have seen a potentially dangerous escalation in chemical warfare. This escalation was symbolized by the Reagan Administration's decision in February to build a new nerve gas munitions plant in Arkansas, approved by the Senate at the end of May. US stockpiles of nerve gas already stand at well over 10,000 tons and while Warsaw Pact stockpiles certainly exist, their quantity is not known. France also has the weapons. The United States is known to have been pressing for a British contribution to its build-up. whether by stock-piling US weapons or other means, even though our government continues to endorse the policy which led to the disposal of the British stockpile in 1957 and the decision not to develop a replacement. Chemical warfare is prohibited by the Geneva protocol and customary international law.

A new chemical arms race in Europe would be an additional hazard over and above that already presented by nuclear weapons Furthermore, it would impose new military direction and priorities on research and development in the life sciences and

biotechnology. Currently, the Ministry of Defence is in contract with British universities to the tune of £5 million per annum. With bleak prospects in front of them, and the reduced level of funding by the research councils, university departments may be tempted to seek defence contracts and acquiesce to all that these entail.

We therefore call on scientific and technological colleagues not to participate in research associated with the development and production of chemical weapons; to urge the British government to forgo the production and stockpiling of chemical weapons in the United Kingdom; and to press the government

- (1) Withdraw its reservation of the right to retaliate in kind made by Britain when ratifying the 1925 Geneva Protocol.
- Resubmit the draft Chemical Weapons Convention tabled by Britain in 1976, revised to incorporate new proposals on verification, consultation, scope and confidence-building measures.
- (3) Promote specific negotiations on the withdrawal of chemical weapons from both sides of Europe.

A significant start in this direction has already been made, namely the support of 2,000 colleagues, including five Nobel laureates, in science, technology and medical faculties throughout Britain. Further support would be welcome.

> SEAN MURPHY ALASTAIR HAY JULIAN PERRY ROBINSON

Open University, University of Leeds and University of Sussex, UK

#### Cancer monoclonals

SIR — The use of monoclonal antibodies in the treatment of cancer is at present being assessed and there is a section of the biomedical community, both scientific and administrative, that believes empirical clinical trials of monoclonal antibodies and toxin-antibody conjugates should be conducted immediately. I wish to present the arguments for a more orderly, scientific and cautious approach.

Three major points can be made in support of the latter case. The first is scientific: it is important to gather as much information as possible before clinical trials with monoclonal antibodies about the tissue distribution of the target antigen, chemical structure and so on. This is for two reasons. (1) The factors involved in either the success or failure of serotherapy cannot be assessed without this basic information. (2) The potential risks or benefits to the patient cannot be accurately measured - this is illustrated by two recent publications. The first reported the (unsuccessful) use of a monoclonal antibody against CALLA antigen in the serotherapy of acute lymphoblastic leukaemia1; the second that the CALLA antigen was not confined to lymphoid cells but was present on several other tissues, particularly in the kidney.2.

The second argument is ethical. The minimum right of a terminally ill cancer patient subjected to experimental therapy is that every effort has been made to assess the potential deleterious effects of the proposed therapy. Moreover, it can be argued that the

patient should also expect the potential benefits of the treatment to outweigh the

The final argument against empirical therapeutic trials of monoclonal antibodies before extensive preclinical testing is that of the relationship between the biomedical community and the general public. In the past, cancer patients and their families have had their expectations of more effective treatments for cancer raised unrealistically by the early optimistic reports of clinical trials with transfer factor, BCG and, more recently, interferon. The biomedical community would be well-advised to avoid a similar situation with monoclonal antibodies.

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#### Room for Anon

SIR - I see that you have again used the word "culprit" in connection with users of the pseudonym Isadore Nabi (Nature 29 October, p.696).

Although I refrained before, I would now like to express my conviction that the use of a pen-name, in publishing a work of literature or science, is not of itself a culpable act. Indeed, the dangers would seem to be least in theoretical science, where such contributions can normally be judged on their intrinsic

As your readers may already be familiar with literary examples, such as that of the Brontes, I will recall a few examples from mathematics.

- (1) In statistics, "Student's test" is so known because it was originally published over the pen-name "Student". The author's true name is known to a few historians. Who says he did anything wrong?
- (2) In Annals of Mathematics (69, 247-251; 1959) we find a letter purporting to come from a mathematician long since dead, saying how glad he is to see his results discovered independently by the mathematicians of the present day. The letter convinces me of the present day author's standing both in mathematics and in the history of mathematics; and he chose an amusing way to make his point. Who says he did anything wrong?
- (3) The pen-name "N. Bourbaki" is used by a French mathematical syndicate, who have published some very useful books and gained a considerable reputation.
- (4) There is some tradition that serious mathematicians, if they write an occasional piece which is recreational or humorous, may shelter behind pen-names such as "H. Petard"

Your mathematical readers can find out which journals I help to edit. I would like to assure them that I shall treat pseudonymous contributions just like any other contributions - on their merits.

J.F. ADAMS

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# Fast breeder reactors: can we learn from experience?

#### Otto Keck\*

INITIALLY, developing the fast breeder reactor (FBR) carried the promise of a virtually inexhaustible source of electricity at competitive cost. Now, three decades and substantial amounts of public expenditure later, we are not much closer to the fulfillment of that promise. Economic evaluations have consistently proved too optimistic. The commercial use of FBRs has been pushed back year after year. This experience gives little comfort to policymakers who have to decide about the next steps in FBR demonstration and commercialization.

Can we identify the sources of overoptimism and improve future policymaking? A close examination of the West German FBR programme, drawing on interviews with about 40 participants and the documents of government advisory bodies, shows a way to bring more realism into policy decisions1. The findings suggest that governments can redress the optimistic bias by requiring reactor manufacturers and utilities to make an increased contribution to the cost of the programme from their own funds. In particular, construction of a commercial-size demonstration plant should be made contingent on the utilities' willingness to finance it from their own funds. Does this proposal take due account of the potential long-term benefits to be expected from the FBR? An economic analysis of FBRs suggests that the answer is yes.

#### Precarious beginning

In West Germany, the development of the FBR began late compared with other countries. The principle of nuclear breeding was discovered in the 1940s, during the United States' effort to develop the atomic bomb. In 1951, a small American FBR produced the first nuclear electricity. By that time the Soviet Union and Great Britain had started on their own programmes.

The initiative for the West German programme came from the Karlsruhe nuclear research centre, a laboratory founded in 1956 with the objective of constructing and operating the research reactor FR2. Although industry contributed about half the construction costs of the reactor, the laboratory was basically

a government organization. When the design of the FR2 reactor was nearing completion, the Karlsruhe scientists looked for new tasks. New work was particularly urgent for the nearly 100 scientists and engineers in the technical division, who faced lay-off after completion of the FR2 reactor. The scientists' interest focused on the FBR and a project group was established in April 1960.

programme the benefits of Euratom money without taking away domestic control. Similar contracts were made in support of the French programme begun in the late 1950s, and of the Italian programme, for which the Euratom contract served as a primer. For the Karlsruhe team, the association implied authorization of an expenditure of DM 185 million (£16.6 million), extending the

The experience of the West German fast breeder reactor programme suggests ways of bringing more realism into governmental decisions on the development of new reactor types. In particular, reactor manufacturers and utilities should finance commercial-size demonstration plants from their own funds.

A preliminary programme for three vears costing DM 25 million (£2.1 million at 1960 exchange rates) was endorsed towards the end of 1960 by an advisory committee of the then Ministry for Atomic Energy. When presenting its plans, the Karlsruhe team referred to American and British forecasts that FBRs would assume a significant commercial role soon after 1970. But the advisory committee by implication rejected these forecasts when it stated explicitly that work on FBRs might be terminated after the preliminary three year programme. This cool reception came mainly from committee members with industrial backgrounds. Ministry officials were very much in favour of the project and the DM 25 million was only a fraction of what the ministry was prepared to spend for a rapid expansion of the Karlsruhe laboratory.

#### **Boost from Euratom**

The threat of possible termination did not worry the fledgeling Karlsruhe project for long. Coincident with the advisory committee's cool appraisal, the European Atomic Energy Community (Euratom) began to show interest in FBRs. The community had been founded in 1958 by Belgium, France, West Germany, Italy, Luxembourg and the Netherlands. It had little success in associating itself with national programmes for first-generation nuclear power plants (light-water reactors [LWRs] and gas-graphite reactors). To secure a useful function, Euratom committed its considerable funds to exotic technological niches, such as the organiccooled reactor, and to advanced designs, such as FBRs.

In 1963 the West German government signed an association contract with Euratom that gave the German FBR project up to 1967. Euratom was to contribute 40 per cent of these funds. This commitment made FBRs the main objective of the Karlsruhe laboratory and gave this reactor type the first priority in the West German reactor programme. In the period 1956–67 — before the first commercial orders for LWRs were placed — FBRs received more government support than any other reactor type.

This shift in priority was made without a fresh consideration of the economic need. For the Karlsruhe project, the Euratom association was a matter of survival. The Ministry for Atomic Energy was motivated mainly by the political aim to secure a fair return from West Germany's contributions to Euratom. In favour of this, it ignored the earlier cool appraisal of the FBR by its advisory committee.

#### Prototype design

In September 1964, the Karlsruhe team was shocked by the announcement by General Electric (US) that the company would offer a commercial FBR as early as 1974. The scientists felt that the West German programme must be pushed ahead as fast as possible to meet this competitive threat. They therefore proposed that work should start immediately on the detailed design of a 200–300 megawatt prototype plant two years earlier than previously planned.

At that time, the laboratory was working on two different versions of the FBR—one using sodium as coolant, the other steam. As a choice between the two seemed difficult, the scientists proposed designing and building one prototype plant of each type. The Ministry for Scientific Research, successor to the Ministry for Atomic Energy, awarded design contracts in November 1966 to two industrial consortia. It allocated DM 57.5 million

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(£5.1 million) for industrial work on the sodium-cooled prototype and DM 38.7 million (£3.5 million) for the steam-cooled prototype.

Apart from the threat of international competition, Karlsruhe's justification for accelerating the programme rested on estimates of electricity costs suggesting that, in the 1970s, FBRs would produce electricity for 15 per cent less than LWRs. The advisory committees did not discuss either argument explicitly. Implicitly the arguments were rejected when reactor manufacturers and utilities declined to make a contribution to the programme from their own funds, arguing that the economic use of FBRs was not foreseeable. Nevertheless, the ministry adopted the Karlsruhe estimates.

#### Termination of steam breeder

At the end of 1967, the industrial team working on the steam-cooled prototype discovered technical problems that prevented conversion of a small boiling-water reactor into a facility for testing FBR fuel elements. When plans for a 50 megawatt steam-cooled FBR in the United States were dropped in 1968, the ministry acknowledged that the ensuing lack of an information exchange with foreign programmes would increase the risks and costs of its own programme. It therefore decided in 1969 to abandon the steam-cooled FBR.

At first, the firms involved opposed termination. However, the ministry got their consent by asking them either to finance part of the further development costs or to agree to the termination. The firms preferred to spend their money on other projects.

#### **SNR-300**

The economic assessment of FBRs reached a turning point in West Germany when contracts for the 300 megawatt sodium-cooled prototype plant SNR-300 were negotiated. Quotes for construction cost were four times more than earlier estimates (excluding inflation). Fuel fabrication costs were quoted as about ten times the previous estimates for commercial FBRs. This ruled out any possibility of FBRs undercutting the electricity costs of LWRs as long as uranium from low-cost reserves was available.

A new economic assessment by an advisory committee concluded that global low-cost uranium reserves might be depleted around the year 2000, so that FBRs would not be needed before the 1990s. Probably two demonstration plants would have to be built after SNR-300 to bridge the gap to commercialization.

Postponement of the SNR-300 might have saved money by shortening this gap. However, changes in the schedule were difficult because the programme had acquired institutional momentum. A large proportion of the Karlsruhe laboratory as well of supplier firms was engaged on the FBR. If the teams were to be kept together, construction of the SNR-300 had to start within a relatively short time.

Construction began in 1973 with only two years delay against an earlier schedule. Belgium and the Netherlands participated in the project, each contributing a 15-per cent share. In 1973, the SNR-300 was

reckoned to cost DM 1,335 million (£205 million), excluding the plutonium fuel. Operation was planned to begin in 1978. Since then, costs have more than doubled (in constant prices). The latest estimate as of February 1981 gives a figure of DM 5,000 million (£1,050 million). Completion is now expected in 1986.

Nearly all the funding for the project has been and is being provided by the three governments. The utilities contribute about 8 per cent of the construction cost from their own funds. West German manufacturing firms finance 8 per cent of the related research and development they perform. The total cost of industrial research and development for the SNR-300, which is not included in the above figures, is estimated at DM 350 million (£75 million). Recently, the German utility participating in the project promised to contribute an additional DM 31 million both in 1981 and 1982, and the German reactor manufacturer pledged an amount of DM 10 million in each of these years. Even so, the firms' contributions to the overall project cost will still be less than 10 per cent. The governments also pay for all research and development performed in government laboratories. For West Germany, this implies an additional estimated expenditure of DM 950 million (£200 million) over the period 1973-82.

#### Lessons

The West German experience suggests that government laboratories and agencies are ill equipped for economic assessments. As they are remote from the commercial world, they may misread the information provided by industry or the technical literature. Organizational needs or political considerations often conflict with a realistic economic evaluation — and when they do, government organizations are tempted to opt for the former.

Reactor vendors and utilities have consistently made more realistic economic assessments than the governmental participants. Yet only at the outset did representatives of industry in the advisory committees volunteer their scepticism, and even that was prompted by the general dispute on the division of responsibilities between government laboratories and industry. Later, firms revealed their scepticism only when asked to contribute some of their own funds. Then they did so implicitly rather than explicitly, revealing it only to the extent that was necessary to defend the limits on their willingness to put up their own money.

As long as only government money is at stake, industrial participants in joint projects seem to have little incentive for a sober review and frank exposure of commercial and technical uncertainties. Criticizing economic assessments made by other organizations is unpleasant. Firms do not wish to exclude themselves from a government-financed programme by questioning the government's economic

West German FBR SNR-300 at Kalkar



justification. Indeed, they want to be part of the programme to know what is going on and to get some of the business.

These conclusions support the proposal<sup>2</sup> that the construction of commercial-size demonstration plants should be contingent on the willingness of utilities to finance the costs from their own funds. A nearcommercial framework should be applied that would require reactor manufacturers to back cost estimates by guarantees with respect to performance and cost, hence making them participate in the technical and commerical risks. Although there is a case for a greater role of government in the earlier phases of a development programme, at the demonstration stage government support should be restricted to the sharing of operating risks and to the financing of background work performed by government laboratories.

Whether demonstration plants are financed through taxes or utility bills, in the end the citizen will have to defray the cost. But the government is likely to get more realistic advice if it asks utilities whether they are prepared to charge the full cost to their customers' electricity bills.

#### Long-term benefits

Against this proposal it may be argued that the FBR may become an indispensable source of energy at some time in the future that is further away than the short-term calculations of utilities can accommodate. A demonstration plant has to be constructed soon, the argument goes, if we are to have the capability of constructing a larger number of FBRs ten to twenty years from now, when there may be a need to do so because of the depletion of uranium resources. A study by the Organisation for Economic Cooperation and Development (OECD) and the International Atomic Energy Agency (IAEA) estimates that at a cost up to \$130 per kilogramme there are about 5 million tonnes of uranium available in the non-communist world (reasonably assured and estimated additional resources). According to the growth projections for nuclear power by the International Nuclear Fuel Cycle Evaluation (INFCE), this may not satisfy demand beyond the year 2010.

But such calculations do not justify paying the penalties involved in constructing a FBR demonstration plant that is more expensive than what a utility would be willing to pay. Uranium prospecting and exploration have a very short history, and vast areas remain to be investigated. On the basis of indirect indications and of geological extrapolations, another study by OECD and IAEA has suggested that an additional 7-15 million tonnes of uranium deposits may be discovered with existing exploration techniques. These may carry nuclear power to the middle of the next century, if a modest growth of nuclear power is assumed.

Past projections of uranium supply and demand have proved an unreliable basis for



French FBR "Super-Phénix" at Creys Malville

policy decisions. Forecasts of future nuclear capacities have steadily been downrated, while estimates of uranium resources have continuously been increased. The lower of two growth projections for nuclear power published by INFCE appears unrealistic only one year later. We do not know how long this trend will continue. The nuclear industry in most countries is presently more concerned about its survival over the short term than about the long-term supply of uranium.

Given the large uncertainties in the longterm demand for uranium and the usual conservativism in resource estimates, there is no assurance that depletion of low-cost uranium resources will necessitate the use of FBRs within the next fifty years. Thus a prudent policy will take into account that depletion of uranium resources will not occur suddenly. As low-cost resources become scarce, the price of uranium may eventually rise to a level that may make the use of FBRs an economic proposition. Uranium resources are likely to be available in sufficient quantity for a transitional period until FBRs replace current types of nuclear reactors. Because of the long lead times of nuclear plant construction, the demand for uranium two to three decades ahead can be fairly easily assessed. The penalties for missing the right time for a transition to FBRs by a decade or two is rather small, as the cost of natural uranium accounts for less than 10 per cent of the total electricity costs in LWRs.

Once reprocessing is available on a larger scale, advanced core designs may become feasible for the LWR that greatly enhance its fuel efficiency. A study by Kraftwerk Union and the Karlsruhe laboratory, for example, proposed a core that uses five times less fuel than present designs. If such designs have lower electricity costs than FBRs, they may delay the need for FBR commercialization by decades.

Premature construction of an uneconomical FBR demonstration plant may also be advocated as an insurance against short-term interruptions of uranium supply. However, one FBR demonstration plant alone would make a negligible contribution, and construction of a series of uneconomical FBRs may provide the desired insurance at a much higher price than other options. A fully developed LWR fuel cycle is little affected by interruptions of two or three years. Longterm supply-contracts can greatly increase the reliability of the international uranium trade. If additional insurance is desired, stockpiling and preparations for emergency mining of low-grade domestic ores or for extracting uranium from seawater may be less expensive options than a series of uneconomical FBRs. Once the technical feasibility of advanced LWR cores is demonstrated and reprocessing is available on a larger scale, this technology will be a strategy, as virtually no insurance premium cost will have to be paid against the threat of a uranium shortage.

#### Economic uncertainties

The perspective that the SNR-300 throws on FBR electricity costs suggests that economic uncertainties are several and large. According to the latest estimate in February 1981, the SNR-300 costs about seven times more per kilowatt of net electric output than a commercial LWR, which was offened at that time at a price of about DM 2,550 (£540) per kilowatt (including owner's cost, but excluding the first core and the interest, inflation and taxes during construction). Scaling-up to sizes around 1,300 megawatt would reduce the cost per killowatt of the breeder by 40-60 per cent. But this would still imply FBR capital costs 2.7 to 4.3 times greater than LWR costs.

The firms constructing the SNR-300, nevertheless, argue that this plant may not be representative of future large FBRs. In particular they say that:

 The cost includes a good deal of research and development that will not have to be repeated for future plants.

- The SNR-300 design is sub-optimal with regard to cost, since design changes imposed by the licensing authorities had to be incorporated into a largely fixed plant concept.
- The supplier's engineering capacity was underutilized as it could not be deployed for other projects during the construction delays caused by the licensing procedure.

These arguments suggest that by extrapolating from the SNR-300 data we may overstate the construction cost of future large FBRs. But we do not know by how much. If engineering services are excluded from the SNR-300 cost estimate, my calculation still suggests FBR capital costs at least double those of the LWR. On the other hand, the costs of SNR-300 are bound to increase further before the plant is completed. As for the next few large FBRs, it is highly probable that most of the factors that have increased the cost of the SNR-300 will still apply, although perhaps to a lesser extent. These factors will become insignificant only if a series of nearly identical plants is constructed at the rate of about one a year.

The hopes of the FBR community now rest on the French 1,200 megawatt Super-Phénix, which costs about twice as much as a French LWR plant of the same size. FBR advocates expect that cost-saving design changes, a relaxation of safety requirements and series production will help reduce construction costs. However, experience with the LWR suggests that it is not certain that cost savings through learning and series production will be quickly achieved. LWR costs have increased dramatically over the past decade. The effects of learning and series production have been cancelled out by other factors such as changes in licensing requirements, increasing quality assurance and control, and schedule slippages.

Estimated fuel-cycle costs for the SNR-300 are so high that the plant would not be commercially competitive even with nil investment costs. According to a forecast in 1973 by the utilities owning and operating the plant, operating costs will be such that the net returns from the sale of electricity are just about enough to pay usual depreciation and interest on a capital investment of DM 100 million. This is less than the amount to be invested in the reactor core (fabrication and plutonium), which for the SNR-300 is paid for by the state, but in commercial operation would have to be financed as a fixed cost by the utilities.

Over the past few years, the cost estimates for the SNR-300 fuel-cycle have become even more unfavourable. Fuelfabrication costs have doubled in constant prices, while the fabrication costs of uranium fuel for LWRs have decreased in real terms. Thus the fuel-fabrication for the SNR-300 is now estimated to cost about thirty times more than for LWR fuel. The original estimate for reprocessing excluded the capital cost of the reprocessing facility.

It was based on the assumption that the fuel of the SNR-300 would be reprocessed in the West German WAK plant, but included no allowance for the construction cost of this facility or for the costs of its adaptation for FBR fuel. When exploring the possibility of reprocessing the SNR-300 fuel in foreign reprocessing facilities, the utility operating the SNR-300 received quotes that indicated much higher costs.

The dismally-high cost estimates for the SNR-300 are partly the result of the small scale on which the fuel cycle for this plant operates. But they also reflect soaring cost estimates for reprocessing of spent fuel and for fabrication of plutonium fuel in general. Because of its toxicity, plutonium fuel has to be fabricated in special plants. These plants can also fabricate plutonium fuel for LWRs. But at present they are still at a pilot or prototype scale.

Recprocessing facilities for LWR or advanced gas-cooled reactor (AGR) fuel can accommodate a few FBR fuel elements if these are reprocessed together with a larger number of LWR or AGR fuel elements. As long as the FBR capacity in operation is small compared with that of the LWRs or AGRs installed, this may be sufficient. Because FBR fuel has a higher plutonium content, generates more decay heat and contains more radioactive fission products than AGR or LWR fuel, the large-scale use of FBRs will, however, require either the modification of reprocessing facilities which were designed for AGR or LWR fuel or the construction of special facilities for FBR fuel.

Over the past decade, cost estimates for reprocessing of spent fuel and plutonium recycle have increased to a level that makes such operations commercially unattractive for LWRs at present uranium prices. The uncertainty persisting in these cost estimates is highlighted by the fact that present contracts with British and French reprocessors have basically a cost plus fixed-fee price. The cost of reprocessing will be known with some certainty only after the French and British have begun operating their large reprocessing facilities for oxide fuel now being constructed and when plutonium recycling is possible within a truly commercial framework.

Even then, however, some cost uncertainties will remain for the FBR fuelcycle, because its technology differs from the plutonium fuel-cycle for LWRs. One such difference concerns cooling time. If spent fuel is stored before reprocessing, its decay heat and radioactivity decreases, so that reprocessing can cost less. The West German reprocessing plant for LWR fuel planned for a site in Hessen is intended for fuel with a cooling time of seven years. Shorter cooling times are essential for FBRs, to make effective use of their notential to breed new fuel and to reduce the financial charges on the fuel bound up in the fuel cycle. It may be technically possible to achieve the desired short cooling time, but it is not yet known what

engineering efforts will be necessary and what impact this will have on reprocessing cost. There is a real possibility that these economic uncertainties may come out unfavourably and that a very large increase in the price of uranium may be required to make the electricity costs of the FBR competitive with those of the LWR. This would delay FBR commercialization by several decades or more. In such an event construction and operation of a demonstration plant would require a large subsidy while the demonstrated technology would become obsolete before a larger number of FBRs are constructed.

#### Time-risk trade-off

Construction of a large FBR demonstration plant in the next ten years or so thus reduces only one of several key uncertainties. It will tell us nothing about the future growth of nuclear power or the availability of uranium; and it decreases only a little the uncertainty about fuel fabrication and reprocessing costs, as these services then will still have to be operated on a small scale.

On the other hand, no matter whether a large FBR demonstration plant is constructed or not, every year that passes gives us more information on future demand for nuclear power and the availability of uranium. And if we wait until rising uranium prices make plutonium recycling a commercially viable operation for LWRs. we shall have a much better basis to assess the economics of the FBR fuel cycle. Yet this may take a very long time if long-term storage of spent fuel proves environmentally as acceptable as fuel reprocessing, and the near-term future of nuclear power is based on LWRs operated in a once-through mode without reprocessing. But the rewards of waiting will be great in terms of reduced economic uncertainties.

As the fuel-cycle facilities of the FBR build on technologies of the LWR fuel cycle, there is a clear case of a time-risk trade-off. Constructing an FBR demonstration plant in the next ten years or so will involve much higher technical and commercial risks than doing the same thing later. And the analysis of uranium supply and demand suggests that there is no commensurable benefit to the public from taking the high risk now rather than taking the much lower risk later. If an FBR demonstration plant has higher costs than utilities are willing to pay, and if risksharing by the government is not sufficient to bring the commercial risk down to a level acceptable to the utilities, subsidies from the taxpayers' purse would be used only to take an unnecessary risk. In such a situation, the short-term calculations of utilities are likely to lead to a decision that is consistent with the public's long-term interest.

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#### NEWS AND VIEWS

### Secondary structure and evolution of ribosomal RNA

from Richard Brimacombe

THE DNA sequencing boom has had an enormous impact on many areas of biochemical research, and the ribosome field is no exception. A series of complete rDNA sequences is now available, and this has led not only to the construction of convincing secondary structure models for the corresponding rRNA molecules, but also to the realization that these secondary structures, as well as significant regions in the primary sequences, have been conserved to a remarkable extent throughout evolution.

The large ribosomal RNA molecules vary considerably in size from one organism to another. At the lower end of the scale are the small mammalian mitochondrial species, with rRNA molecules of 12S and 16S in the small and large subunits (approximately 950 and 1,550 nucleotides in length, respectively). In the middle are the ribosomes from bacteria, together with chloroplasts and other types of mitochondria, containing rRNA molecules of 16S and 23S ( $\sim 1,550$  and 3,000 nucleotides) and at the upper end of the scale are the eukaryotic cytoplasmic ribosomes with rRNA molecules of 18S and 26-28S ( $\sim 1,800$  and up to 4,000nucleotides, respectively).

#### Sequencing

The first rDNA sequence, that of 16S rDNA from Escherichia coli, was completed only three years ago1. Now the list of available sequences includes the 23S rDNA from E. coli<sup>2</sup>, the 16S and 23S rDNA from maize chloroplasts<sup>3</sup>, the 12S and 16S rDNA from human mitochondria4 and mouse mitochondria<sup>5</sup>, the 15S rDNA from yeast mitochondria6, and the 18S rDNA from yeast<sup>7</sup> and Xenopus laevis<sup>8</sup>. Further sequences were reported at the recent EMBO Symposium on Ribosomes\*, including the 23S rDNA from Bacillus stearothermophilus (Noller, Santa Cruz), 15S rDNA from Aspergillus nidulans mitochondria (Köchel, Göttingen), and the 26S rDNA from yeast (Planta, Amsterdam); the 28S rDNA from Xenopus is well on the way to completion (Gerbi, Providence). Partial sequences from a host of other organisms have also been described, and in addition the complete 16S molecule from Proteus vulgaris has been

worked out by direct RNA sequencing methods9. In short, we now have examples of ribosomal RNA or DNA sequences from all the principal categories mentioned above, with the notable omission of an archaebacterial map.

#### Secondary structure

In order to get from primary sequence to structure, sequence secondary comparisons have been combined with various experimental approaches based on data derived from E. coli 16S and 23S rRNA. Three groups, in California, Strasbourg and Berlin, have been principally concerned in these studies, and each group has independently constructed models for the secondary structure of the ribosomal RNA from both subunits. The experimental approaches have been (1) the analysis of sites of chemical modification by single-strand specific reagents (California), (2) the analysis of sites of clipping by single- or double-strand specific ribonucleases (Strasbourg), and (3) the isolation and analysis of short basepaired fragments of the RNA, together with the determination of sites of intra-RNA cross-linking (Berlin).

The various possible secondary structural features suggested by these experimental data can then be tested by sequence comparisons, in order to derive the correct structure. This sequence comparison approach, which was successfully applied some time ago to the case of 5S rRNA by Fox and Woese<sup>10</sup>, is in essence very simple. If two very similar but not identical sequences from different organisms are compared, then a base change in one strand of a putative doublehelical region must be compensated by a complementary base change in the other strand. Thus, an A-U base pair in one species could become a G-C pair at the corresponding positions in the second species, and so on. If the base changes between the sequences do not compensate each other in this way, then the implication is that the proposed element of secondary structure is incorrect. The secondary structure models for 16S and 23S rRNA have been successively refined by this approach, the latest versions (for the small subunit) being by Noller and Woese11, Stiegler et al. 12 and Zwieb et al. 13, for the the large subunit, the Berlin and

Strasbourg groups recently published structures almost simultaneously14.15, whereas the model from the Californian group is still in the final stages of preparation.

The secondary structure models for both subunits are in about 80 per cent agreement with one another. Although there are still some important differences, the main features, that is, the 'long-range interactions' between RNA segments widely separated in the primary sequence and which serve to divide the molecules into well defined structural domains, are firmly established. It is also clear that all molecules in the same size class (16S or 23S rRNA) can be arranged in almost identical secondary structures, and the extent of primary sequence homology within this class is very high; maize chloroplast rRNA is, for example, 70 per cent homologous with E. coli rRNA, and if the two 23S molecules from these species are compared, then over 450 compensating base changes can be found between the proposed secondary structures.

#### Size difference

More exciting, however, is the finding that the structural comparisons can be continued outside the 16S and 23S rRNA size classes both to smaller and to larger rRNAs. The Berlin and Strasbourg publications cited above show that the small mammalian mitochondrial rRNA molecules (12S and 16S) contain many elements of secondary structure precisely equivalent to their 16S and 23S counterparts. Reduction in size is achieved by simple amputation of secondary structural loops, or by erosion of a whole domain. Recognition and location of secondary structural features is made easier by searching for stretches of conserved primary sequence, which tend to be - but are by no means exclusively - in singlestranded regions. There are, however, some interesting anomalies, where apparently significant stretches of homology have 'leap-frogged' each other in the different sequences. Precisely the same situation pertains if one makes a comparison with the longer eukaryotic

Richard Brimacombe is in the Max-Planckthree groups, respectively. In the case of Institut für Molekulare Genetik, Berlin-

Dahlem.

cytoplasmic rRNA molecules (see also ref. 16). The same secondary structural features are conserved, and the extra sequences in the eukaryotic molecules are accomodated in precisely those regions which are shortened in the case of the small mitochondrial species. Again, there is striking conservation of primary sequence in both single- and double-stranded regions. The secondary structure comparisons also confirm the suggestions (based on primary sequence homology) that eukaryotic 5.8S rRNA is equivalent to the 5' end of prokaryotic 23S rRNA17, and that the 4.5S rRNA from chloroplasts is correspondingly equivalent to the 3'end of the bacterial 23S rRNA18.

One should not be too surprised by these findings; after all, both tRNA and 5S rRNA have been highly conserved throughout evolution. What is important is that the extension of the conservation to the large ribosomal RNA molecules of all classes has now been clearly demonstrated, and that this conservation will per se be a powerful tool for testing the validity of any new structural or functional proposals in the future.

glaciology is whether or not the West Antarctic Ice Sheet (WAIS), as well as the East Antarctic Ice Sheet, is stable. It is suspected that it is inherently unstable because its base is far below sea level for most of its area. A related question is whether the expected increase in atmospheric CO, and the climatic warming it may produce will propel the ice sheet to its destruction.

#### Stability

The symposium opened with sessions addressed to the problem of ice sheet stability. Because most of the ice of the WAIS is drained through ice streams, the latter and their relationship to stability received much attention. One way to find out whether the WAIS is growing, is slowly disintegrating or is in a steady state is to see if the grounding lines where they meet ice shelves are stationary. British Antarctic Survey (BAS) scientists reported the first ever measurement of the position of an ice stream (Rutford Ice Stream) grounding line made with sufficient precision to determine within the next few years if it is stationary or not. We should not have to wait too long to learn whether the WAIS over the next century is benign.

Ohio State and Grenoble Universities glaciologists used indirect evidence of the volume of air in ice from the Byrd Station bore hole to conclude that the WAIS has been rather stable during past major climate and glacial changes. It has been stressed by University of Maine researchers that the Pine Island Glacier is where the WAIS may collapse but studies by BAS glaciologists have found no evidence of instability - although their findings cannot rule out its existence. The last 100 km of the Pine Island Glacier were found to be afloat and to be essentially a onedimensional ice shelf. The existence of this ice shelf separates the calving front from the grounding line and probably increases

#### Carbon dioxide

stability.

Whether a carbon dioxide build-up will make the Antarctic ice more or less stable is not certain. One definite piece of evidence was reported by University of Bern geochemists. The data, reconfirming their earlier work as well as that obtained at Grenoble, show that the CO<sub>2</sub> content of the atmosphere during the last ice age was lower than it is now. The measurements were made on entrapped air within ice cores brought up from deep within the ice sheets but whether the low CO, content is the cause, or is the effect, of an ice age is still anyone's guess. If a low CO, content and a large ice volume go hand-in-hand, then it is possible that a high CO, level could cause at least the West Antarctic ice to decrease in volume. A process that is likely to produce

\*The Third International Symposium on Antarctic Glaciology was held at Ohio State University, Columbus, Ohio, 7-12 September 1981. The proceedings will be published as a volume in the Annals of Glaciology.

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#### On Antarctic glaciology: Ice sheets. .

from J. Weertman

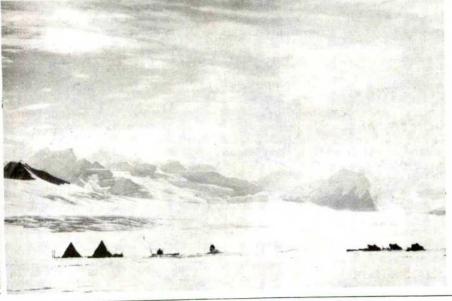
An international symposium on Antarctic glaciology\* was held this autumn at Ohio State University. It was only the third meeting on this topic in over twenty years and it might be supposed that progress in this field moves at the proverbial speed of a creeping glacier. Actually, the pace of research has accelerated greatly in keeping, perhaps, with the recent discovery that the major glaciers in Antarctica, namely the great ice streams, move with impressive velocities.

Results were reported from research projects carried out in virtually all parts of the Antarctic continent, in oceanic islands

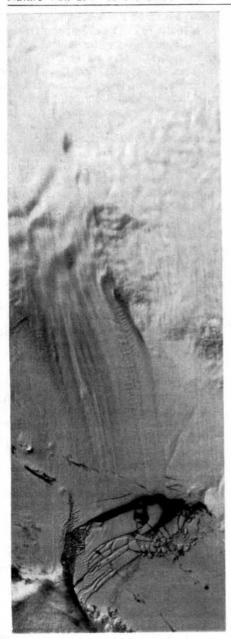
near the continent, and on the sea ice that surrounds it. The topography of the Antarctic ice sheets has been partially surveyed from satellites, with an elevation error of about 1.5 m, and the ice sheets have been probed by deep drilling (most recently by a 900 m core hole at Dome C in East Antarctica), by seismic sounding and by radar sounding. Data were obtained that describe in an increasingly quantitative manner the present ice mass and some of its past history and offer clues to its future behaviour.

The most interesting problem that remains to be solved in Antarctic

Field camp on the Rutford Ice Stream with the Vinson Massif in the background.



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A NASA Landsat picture of the Pine Island Glacier. At the top of the picture the glacier ice is more than 2,000 metres thick but this falls to 500 metres where icebergs are calving off into Pine Island Bay at the bottom of the picture.

a reduction in ice volume is an increase in the rate of calving which would eventually lead to the destruction of the ice shelves and of the ice sheet they fringe.

#### Warming effect

The calving process is not well understood but a big advance was reported by University of Maine scientists who used rather detailed computer modelling calculations to study the process. We are still a long way though from predicting what would be the effect of, say, a 1°C warming produced by a CO<sub>2</sub> increase on the rate at which icebergs are created in Antarctica.

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SINCE THE 1960s, when it was recognized that a chronological history of the composition of the atmosphere is contained in the Antarctic ice sheets, ice drilling has featured strongly in most national programmes. Analysis of ice cores is now making it possible to reconstruct variations in past climate, global volcanic activity and atmospheric pollution. The symposium provided an opportunity to review progress in these areas.

Particularly dramatic has been the evidence from deep ice cores of changes associated with the transition from the Wisconsin (Würm) glaciation to the Holocene. Lorius (Laboratoire de Glaciologie, Grenoble) discussed new data from the 905 m Dome C core which support earlier reports by Thompson (Institute of Polar Studies, Ohio State University) from this and the Byrd core that the number of microparticles increased by a factor of eight, relative to Holocene values, during the last glaciation.

But to what extent is the inverse relationship between increased particle load and temperature causal? Lorius Glaciologie, Grenoble) has detected the influence on sulphate levels of several major volcanic events in both recent firn and in ancient ice back to 30,000 years BP. The input of sulphate from volcanic sources is only significant during the three to four years following an eruption. Such studies will provide an accurate record of volcanic activity over periods of tens of thousands of years and should also provide a powerful method for dating ice cores.

However, there is still a substantial input of sulphate in volcanically quiet periods which cannot be accounted for by input of marine salts. Delmas supports the idea that it originates in part from the oxidation of biogenic gaseous sulphur compounds, such as DMS, at the ocean surface. The aerosol may then be transported to the polar plateau through the upper troposphere. On the other hand Herron (State University of New York, Buffalo) has found clear seasonal variations of sulphate with summer maxima which do not correlate with marine chloride. Thus he believes a non-marine gaseous sulphur compound is the most likely source, with

#### . . . and ice cores

from David A. Peel

argues that a global increase in arid areas during the last glaciation combined with a fifty per cent increase in wind speed associated with a strengthened meridional circulation would be sufficient to account for the enhanced microparticle load. In the sections of core analysed by his team he could find no evidence for increased volcanic activity in the glacial period either by examination of the larger particles, or in the levels of gas-derived sulphate. However, Thompson has clearly identified bands of large (60-80 µm) volcanic glass fragments in other segments of the same core and found they occurred with greater frequency in the Wisconsin. Clearly, a means must now be sought to distinguish whether dust in the smaller and more abundant size ranges is volcanic or continental in origin. Millar (Scott Polar Research Institute, Cambridge) gave evidence that radio-echo sounding can detect the acid layers associated with volcanism and provides a means for deducing broad changes in the level of volcanic activity over periods of tens of thousands of years. This type of study could, in the future, rapidly extend knowledge previously based on the much slower analysis of ice cores.

The major impurity in Antarctic ice is sulphate yet the mechanism by which it reaches the Antarctic ice sheet is largely unexplained. Application of new methods has allowed much more detailed sampling from a wider area than before. In the Dome C ice core Delmas (Laboratoire de photooxidation of the gaseous precursor

photooxidation of the gaseous precursor playing a significant role. A clear inverse relationship between snow accumulation rate and sulphate concentration at sites in both Greenland and Antarctica further indicated dilution by snowfall of aerosol derived from a gaseous precursor in the upper troposphere or lower stratosphere.

#### Nitrates

Nitrates in snow are presenting greater difficulties. Zeller has made extensive measurements on 100 m cores and shallow pits at South Pole and Vostok station which support his earlier evidence that much of the NO<sub>1</sub>-N in Antarctic snow is derived by auroral fixation of NO, and follows an 11 year cycle in solar activity. Elevated NO<sub>1</sub>-N levels coincided with years of solar flare activity peaks which are associated with high auroral activity. Pronounced seasonal variations with maxima in winter months could be due to summer photochemical destruction of much of the NO. The broad, longer term changes at the South Pole and Vostok were similar with prolonged, low NO3-N concentrations at the time of the Maunder minimum (1617-1710).

In contrast, Herron, who also included NO<sub>3</sub> measurements from the South Pole in a detailed investigation of the anion content of snow, although agreeing with the mean NO<sub>3</sub> values reported by Zeller,

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observed much less variance in concentration even with a greater sampling frequency and could not detect any deviation during the period of the Maunder minimum or any of the spikes which Zeller attributes to solar flare or supernovae events. Moreover, he detected a seasonal variation with summer maxima in parallel with variations in sulphate. Delmas also found that nitrate concentrations in the

Dome C core have been relatively constant over the last 30,000 years. To complicate the picture even further, in a detailed profile at James Ross Island he found virtually no seasonal variability. In view of the potentially great value in using nitrate to trace past solar fluctuations the conference agreed that high priority should be given to resolving these seemingly incompatible data series.

#### A virus associated with human adult T-cell leukaemia

from Robin Weiss

RECENT virological, serological and epidemiological evidence strongly indicates that a newly discovered retrovirus is the aetiological agent of certain types of adult T-cell lymphoma/leukaemia. The virological evidence first came from R.C. Gallo's laboratory at the US National Cancer Instititute1 where the retrovirus was isolated from a T-lymphoma cell line established in culture from a patient with mycosis fungoides. In this issue of Nature Poiesz et al. (see p.268) report the isolation of a similar virus from a patient with Sézary syndrome, while Kalyanaraman et al. (see p.271) show that the two patients' sera (and that of a patient's spouse) contain antibodies which specifically react with the major core protein of the virus.

Mycosis fungoides and Sézary syndrome are clinical variants of cutaneous T-cell lymphoma and leukaemia, respectively, that occur as rare diseases in adults<sup>2</sup>. The retrovirus particles produced by these tumours are not evident until the tumour cells are grown in culture, although a second biopsy from the first patient released virus within 24 h of its being placed in culture. Until 5 years ago it was very difficult to maintain normal or malignant T cells in culture; however, a specific T-cell growth factor (TCGF) discovered in Gallo's laboratory3 enables T cells to proliferate in vitro for long periods, and this aided the detection of the virus.

#### Properties of HTLV

The virus, called HTLV (for human T-lymphoma virus), seems to be quite distinct from the numerous types of animal retroviruses previously described. By its morphology and the molecular weight of virion proteins, HTLV most closely resembles bovine leukosis virus, which causes lymphoma in cattle. However, the human and bovine viruses seem unrelated antigenically or by nucleic acid homology.

Following Gallo's report of virus production from T-lymphoma cultures, an

independent virus isolate has been made in Japan<sup>4</sup> from a previously established malignant T-cell line5. Now Miyoshi and his colleagues, in a paper shortly to be published in Nature, claim that cocultivation of the T-leukaemia cells with umbilical cord leukocytes leads to the transformation of the cord leukocytes into a TCGF-independent cell line producing retrovirus particles. This is the first experimental evidence for infectious transmission and cell transformation with HTLV.

#### Origin of HTLV

Gallo's laboratory has not succeeded in transforming animal or human cell lines with HTLV, but they have not tested cord lymphocytes nor used co-cultivation. They think that T lymphocytes from some relatives of patients may support the replication of HTLV. At a recent meeting of the Leukaemia Research Fund in England, Gallo reported individual isolates of HTLV from four cutaneous T-lymphoma patients. From one patient an Epstein-Barr virus-positive B-lymphoblast line was also developed: this line was negative for HTLV, suggesting that HTLV is specifically associated with the tumour cells. Serological tests on some hundreds of American normal individuals or patients with diseases other than T-cell malignancies have been negative for antibodies reacting with the HTLV p24 core protein. By no means all T-cell leukaemia patients are positive either; the virus appears to be associated with a particularly malignant, fast growing form of tumour. Gallo thinks that all four patients (three black and one white) from whom HTLV has been isolated have Caribbean connections and that the virus may be more commonly found there.

The most striking epidemiological studies, however, come from Japan. There is a relatively high incidence of patients in major Japanese cities presenting an aggressive form of adult T-cell leukaemia. Uchiyama et al.6 noted that 13 out of 16 patients with this form of disease were born and grew up on the west coast of Kyushu, Japan's extreme south-west island. A more recent study<sup>7</sup> of 272 T-cell leukaemia cases shows a remarkable clustering of places of birth, in the Kagoshima and Nagasaki prefectures of Kyushu. Sera from all the leukaemia patients tested and from 25% of healthy adults sampled react positively with Miyoshi's virus isolate4. Through Yohei Ito, Gallo has found that Japanese sera also recognize his viral p24 antigen.

Several viruses are implicated as aetiological factors in human malignancies; Epstein-Barr virus in Burkitt's lymphoma and nasopharyngeal carcinoma are the classic examples. The association of hepatitis B virus infection with liver cancer has become apparent although it is curious that this virus does not even merit passing mention in two major compendia on tumour viruses published as recently as 19808.9. Cancer of the uterine cervix may well have an infectious agent as an aetiological factor, for which herpes simplex and papilloma viruses are under suspicion. During the 1970s claims for isolating human retroviruses have perhaps been made too frequently and prematurely. While some proved to be animal viruses acquired during laboratory procedures, others remain enigmatic, such as the viruslike particles seen in full-term placentae<sup>10</sup>. Four laboratories — Bentvelzen's in Holland, Gallo's at the National Institutes of Health, Kaplan's at Stanford and Kirsten's in Chicago — have independently isolated from human tissues retroviruses related to those of gibbons and baboons. These isolates are generally dismissed as laboratory contaminations. I am reluctant to disclaim their human provenance so lightly, although admittedly human antibodies which were thought to bind to these viruses specifically now appear to recognize heterophile carbohydrate groups present on the viral glycoproteins<sup>11,12</sup>.

#### Role in malignancy

The striking features of the T-cell leukaemia virus are its unique biochemical properties, its association with a particular subset of T-cell lymphoma/leukaemias and the geographical clustering of that disease in Japan. The discovery of this new virus followed the successful development of culture methods for T cells. The epidemiological findings would not have been possible without careful classification of leukaemias into B- and T-cell classes and further subclasses. A fascinating and important story is unfolding on the viral aetiology of a human malignancy.

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#### Progress on accretion disks

from J. Craig Wheeler

ASTROPHYSICISTS are wrestling with the study of a new kind of star, the flat twodimensional configurations known as accretion disks. Accretion disks exist in a variety of contexts where mass is deposited to swirl around a compact star such as a white dwarf or a neutron star. They are suspected to play a part in more exotic situations, like active galactic nuclei and quasars, where the central object is imagined to be a supermassive black hole. The study of these objects is still in its infancy, analogous to the early days of Eddington when stars were modelled using elementary scaling laws without benefit of knowledge of the nuclear processes which powered the stars. Similarly, the power by which accretion disks radiate is suspected to come from a form of turbulent friction, the basic physics of which is known only at the crudest level. Most of the current literature on accretion disks is couched in terms of parametrized scaling laws which avoid direct confrontation with our most basic levels of ignorance. The letter by Abramowicz in this issue of Nature (p.235) adds needed rigor to our understanding of the structure and evolution of accretion disks while exploring an instability in the disk structure which has plagued earlier models.

Accretion disks were first defined as astronomical entities in the context of cataclysmic variables. In these systems, which include novae, matter from the outer layers of an ordinary star is attracted by the gravitational influence of a nearby orbiting white dwarf star. The matter lost from the ordinary star cannot strike the surface of the tiny white dwarf directly, but settles into nearly keplerian orbit. The viscosity in the disk causes heating and radiation and a slow spiralling of the material onto the surface of the white dwarf.

The rapid advances made in X-ray astronomy in the past decade have identified similar systems in which the accretion disk whirls about a neutron star rather than a white dwarf. The inner reaches of the accretion disk extended deeply into the gravitational potential of the neutron star where very rapid motion is the rule. The energy released by friction and the actual raining of the material from the disk onto the surface of the neutron star is so great that the radiation comes off in a powerful flood of X rays. In at least one binary system explored by X-ray astronomers, Cygnus X-1, the object in the centre of the accretion disk is thought by many to be a black hole, the ultimate form of collapsed matter.

Complete understanding of the out-

bursts of cataclysmic variables and X-ray production by neutron stars demands knowledge of the associated accretion disks. Through such study may also come deeper insight into phenomena such as active galaxies where matter from millions of stars may gather around a gigantic black hole in the galactic centre.

Previous work has shown that portions of accretion disks are unstable, leading to the clumping of matter into rings or bloating into a fat hot doughnut, in contradiction to assumptions that the disks are geometrically thin. However, there is no observational evidence that accretion disks do, in fact, suffer from these instabilities. Abramowicz argues that these instabilities may not occur in reality, and he shows that added gravitational effects due to general relativity alter the potential compared with the standard newtonian case in such a way that the instabilities are removed.

Ironically, this work may have most direct application in the case of accretion disks around supermassive black holes where observational confirmation of the basic picture is weakest. Abramowicz argues that the major inner radiating portions of such a supermassive disk will be stabilized in this case. The relativistic effects of which he speaks should also apply to putative stellar mass black holes, as in the case of Cygnus X-1, but this system is know to undergo some transitions in its radiative properties which have been discussed in terms of disk instabilities. For systems with white dwarfs and neutron stars the surface of the star or the presence of a surrounding magnetosphere may dominate the inner portions of the disk and overwhelm the relativistic effects described by Abramowicz. Even in the case of supermassive black holes there is speculation that the inner region consists of a nearly spherical, differentially rotating configuration, so that the basic assumptions underlying a thin disk analysis do not apply.

This discussion serves to illustrate the complexity of the phenomena known collectively as accretion disks. Progress towards understanding them will involve defining and solving restricted problems just as was done, and continues to be done, for ordinary spherical stars. The work by Abramowicz gives a new valuable example of the care which must be taken before reaching definitive conclusions regarding accretion disks.

#### Geomechanics in the laboratory

from Neville G.W. Cook

THE increasing use of the ground beneath us, whether in mining activities, underground construction or in the disposal of toxic wastes, requires, as does an understanding of earthquakes, a much deeper knowledge of the behaviour and properties of rocks than has sufficed in the past. To understand the behaviour and properties of rock masses, in situ tests are possible but they are costly and time consuming<sup>1-3</sup>. As became clear at a recent conference (see Geophysical Research Letters 8, 1981 for the proceedings) there is a need for laboratory tests on a scale of the same order as that of the in situ tests, both to avoid many of the restrictions of in situ tests and to bridge the gap between them and usual laboratory tests.

The most common kind of laboratory test on rock is the 'triaxial' test first introduced in 1911 (see ref.4). Right circular cylinders of rock, usually a few centimetres in diameter and several times greater in length, are subjected to axial compression between the platens of a mechanical press and an equal radial compression, applied through an impermeable membrane by a confining fluid pressure.

There are fundamental differences in the problems faced by laboratory testing in increasing deformation leads to decreasing

engineering mechanics and in geomechanics. In engineering mechanics, man-made materials are generally used and laboratory specimens can be expected to have similar properties to those of structural members made of the same material. Such materials are relatively simple in composition and structure compared with the composition and structure of most rocks. Again, laboratory test specimens of manmade materials are usually within an order of magnitude of the size of structural members whereas the dimensions of excavations and geological features can be many orders of magnitude greater than those of the rock specimens.

A further problem is that systems comprising conventional, soft testing machines and specimens of brittle rock become unstable at or beyond the peak of the stress strain curve<sup>5</sup>. Until the advent of stiff or servo-controlled testing machines, it was impossible to study the 'worksoftening' deformation of rock, where increasing deformation leads to decreasing

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resistance to deformation. Stable, worksoftening deformation is of great importance in many practical problems, such as the stability of underground excavations and aseismic movement along faults. A final difference is that it is possible to subject specimens of man-made materials to loads similar to those they will experience in use, while small laboratory tests cannot reproduce the effects of stress concentrations in metres to kilometres in a rock mass subjected to a poorly understood state of stress.

Despite these limitations, the triaxial laboratory test has been of immense value in providing scientists and engineers with an understanding of the behaviour and properties of rock. As examples can be cited the transition from brittle to ductile behaviour as a function of confining stress and temperature, the effects of fluid pressure, and the phenomena of dilatation and brittle cracking in compression<sup>6,7</sup>.

The behaviour and properties of most rock masses in situ differ from those measured in conventional laboratory tests on specimens of the same rock. The reasons for these differences are not well understood but it is likely8,9 that size is one

of the factors involved. There are likely to be many others, such as variations in geological composition and structure, on all scales.

At present, three large-scale laboratory test facilities using specimens of rock with dimensions of the order of a metre are in use in the United States. The three facilities have each been designed for a specific purpose - the Lawrence Berkeley Laboratory triaxial apparatus for testing the mechanical and hydraulic properties of jointed rock at low stresses<sup>10</sup>, the US Geological Survey's biaxial frame for testing simulated faults in large blocks for rock<sup>11</sup>, and the Terra Tek Drilling Research Laboratory.

The proceedings of the workshop made it clear that large-scale laboratory testing is likely to be much more costly than is conventional laboratory testing, but such a facility will answer fundamental questions in rock mechanics. Comprehensive measurements of phenomena occurring inside a rock specimen, such as the location of sources of acoustic emission and fracture initiation and propagation, could be made under a wide range of controlled conditions of stress or strain, pore fluid pressure and temperature.

It was pointed out that the simplicity of the triaxial test is deceptive; it, in fact, involves homogeneous axial strain and homogeneous radial stresses. A large-scale triaxial high pressure and temperature cell may be excessively costly and potentially hazardous. A successful large-scale laboratory testing facility is likely to have to be different in concept and design from a conventional triaxial cell.

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#### Morphological stasis and developmental constraint: real problems for neo-Darwinism

from Peter G. Williamson

A recent News and Views article1 commented on a paper of mine2 which summarized a morphometric analysis of mollusc lineages from a Cenozoic sequence in North Kenya. I had made three key points: (1) all lineages exhibit morphological stasis for very long periods of time (3-5 Myr), (2) evolutionary change in each lineage is concentrated in relatively rapid speciation events (occurring over 5,000 to 50,000 years) and (3) the speciation events are accompanied by pronounced developmental instability in the transitional populations.

The first two points indicate that these lineages conform to the 'punctuated equilibrium' model for evolutionary change and the third is significant because the unusually complete Turkana Basin sequence offers the first fine-scale palaeontological documentation of the speciation process.

The News and Views article considers only the second of these three points, making the uncontested observation that geneticists have succeeded in producing significant phenotypic changes in many populations over periods considerably shorter than those required for speciation events documented in the Turkana Başin mollusc sequence. The question of rapidity of speciation events is addressed but no attention is paid to the problems either of long-term morphological stasis or of developmental instability during speciation. Such comments are the standard but largely tangential criticisms advanced by many evolutionists against the punctuational model. The possibility of rapid change is freely admitted, but the significance of stasis, and the implications developmental constraint for evolutionary process, are ignored. Since the critique (and others recently published)3-5 seem to miss the most important issues raised by punctuated equilibrium theory in general, and my own work in particular, some comments seem in

As Gould and Eldredge<sup>6</sup> and many others have repeatedly pointed out, punctuated equilibrium is a theory about the deployment of speciation in time. It holds that many, perhaps most, metazoan fossil sequences show a characteristic pattern of morphological change through time: new species enter the record abruptly (in geological time), and persist with little significant change until extinction. Significant evolutionary change is, therefore, concentrated at speciation events.

For reasons that elude many of us, punctuated equilibrium, a theory of evolutionary tempo, has been conflated

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with Goldschmidtian macromutation, a theory of evolutionary mode. Thus, in the critique of my paper, I am lumped with Goldschmidt (and Cuvier of all people) as disciples of some peculiar (and nonexistent) non-Darwinian evolutionary school. But punctuated equilibrium is compatible with much current neo-Darwinian thought. Eldredge and Gould7, in their original formulation, relied upon the most orthodox version of Mayr's theory of allopatric speciation via peripheral isolates to account for the 'punctuational' pattern of morphological change in the fossil record. They considered the abrupt appearance of new species and their subsequent stasis, as well as the lack of intermediates between such stable lineages, to be compatible with (indeed, to flow from) Mayr's model. Mechanisms for rapid speciation that have been proposed subsequently (for example, the various models for 'chromosomal' speciation) are compatible with but not required by punctuated equilibrium, a theory of evolutionary tempo that is agnostic about modes of speciation, so long as they yield (as most standard models of speciation do) the punctuational pattern when translated into geological time.

It is not news to punctuationists that population geneticists can produce rapid phenotypic shifts in artificial selection regimes within a few generations -

punctuationists and conventional neo-Darwinians are in complete agreement on this point. Why, then, are many punctuationists increasingly unhappy with conventional neo-Darwinian accounts of fundamental evolutionary process?

The principal problem is morphological stasis. A theory is only as good as its predictions, and conventional neo-Darwinism, which claims to be a comprehensive explanation of evolutionary process, has failed to predict the widespread long-term morphological stasis now recognized as one of the most striking aspects of the fossil record. The long-term morphological stasis noted by punctuationists in the fossil record is clearly mirrored by the relative morphological uniformity of most widely distributed modern species. As Ernst Mayr, the foremost student of geographical variation has writtens: '...it would . . . seem important to stress the basic uniformity of most continuously distributed species . . . The fact that (every taxonomist)...can identify individuals of a species . . . regardless of where in the range of the species they come from is further illustration of this phenomenon'. In a belated attempt to address the problem of morphological stasis, neo-Darwinists have invoked 'stabilising selection' (for example, Stebbins and Ayala<sup>5</sup>). But the wide range of environments presently exploited by extensively distributed but morphologically uniform modern species, and the long-term morphological stasis (up to 17 Myr) exhibited by many fossil lineages in fluctuating environments, strongly argues against the idea that simple stabilising selection is an adequate explanation for the phenomenon of morphological stasis. Accordingly, Mayr explicitly invokes some form of developmental homeostasis, rather than stabilising selection, to explain the rangewide morphological stability of most modern species.

In the original formulation of punctuated equilibrium theory, it was suggested that temporal stasis, like the geographic stability noted by Mayr, was largely the result of some form of developmental constraint or homeostasis. In the absence of a comprehensive genetics of development, the mechanism for such homeostasis is obscure. But if some form of developmental homeostasis is at the root of morphological stasis, speciation must, by definition, involve the dismantling of homeostatic mechanisms pre-existing in the parental stock. The principal argument In my paper is that when speciation events occur in the Turkana Basin mollusc sequence, they are invariably accompanied by major developmental instability (that is, just such a dismantling of developmental homeostasis). The idea that morphological stasis is primarily a result of developmental homeostasis, and that speciation must therefore involve the temporary dismantling of such a homeostatic system,

differs from most conventional neo-Darwinian assumptions about the way in which species arise. Most neo-Darwinists would agree with Darwin's statement that new species arise 'solely by accumulating slight, successive, favourable variations'. They believe that the intrapopulation micro-evolutionary changes observed in the Drosophila cage can be simply extrapolated into the differences between Drosophila species and Dipteran families. In this view of speciation, as Gould says, there is a 'seamless continuum' a 'smooth extrapolation . . . from base substitution to the origin of higher taxa'. There is no suspicion here that the fundamental developmental constraints implied by longterm geographical and temporal stasis of species must be dismantled when new species arise. There is no suspicion that this radical reorganization of fundamental homeostatic mechanisms during speciation must involve a more radical overhaul of the phenotype than the steady 'march of metric means' seen in a Drosophila cage experiment.

Interestingly enough, the idea that disruption of developmental homeostasis, or constraints, is central to the speciation process is hardly new to the neo-Darwinian literature: Carson<sup>10</sup> has postulated 'open' and 'closed' genetic systems, the former involved in the alleleshuffling of minor adaptive adjustments within species populations, and the latter involved in the more profound regulatory

and developmental changes during speciation. Levin<sup>11</sup> has pointed out the significance of developmental disruption and instability during the speciation process. But such suggestions have been largely ignored: the implied decoupling of micro-evolutionary change and macroevolutionary phenomenon threatens the reductionist core of conventional neo-Darwinism.

The Turkana Basin sequence records a pattern of long-term stasis punctuated by rapid speciation accompanied by pronounced developmental instability. Jones suggests that this pattern requires no change in conventional views of the genetic mechanisms of the origin of species despite the fact that this pattern is neither predicted by neo-Darwinism nor explicable in terms of its major tenets. Punctuationists suggest that it is time for conventional neo-Darwinism to address the important issues of morphological stasis and developmental constraint.

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#### Life on an oily wave

from A.J. Southward

AFTER three years of deliberation, the Royal Commission on Environmental Pollution has decided that chronic and accidental spills of petroleum do not pose a permanent threat to marine life comparable with the dangers inherent in heavy metals and radioactivity (Nature 293, 692; 1981). This view, with which many scientists concur, is based on the assumption that petroleum is degraded by physical, chemical and biological breakdown, and thus does not have a cumulative effect. Nevertheless, there is still public concern about oil in the environment because it is so evident, whether fouling the shoreline or killing sea-birds. Scientists too are concerned about the extent to which marine communities are disturbed by oil, and how long they take to return to normal. In consequence, a meeting\* was held recently to discuss the long term effects of oil pollution on marine populations, communities and

ecosystems. There was considerable disagreement about the importance of sublethal effects; the length of time needed for recovery; and whether the effects of pollution can be detected when natural communities show extensive short and long term fluctuations in abundance and species composition. However, some concensus was reached that planktonic and pelagic communities have been relatively untouched by oil pollution (J. Davenport. Marine Science Laboratories, University College of North Wales). This and evidence that even badly affected salt marsh communities can begin recovery after improvements to refinery effluents (B. Dicks, Orielton Field Centre, Pembroke) allowed cautious optimism about the future.

The first contribution set the scene by describing the origins and fates of the estimated six billion tons of oil that reaches

<sup>\*</sup>Discussion meeting at the Royal Society, 28–29 October 1981, organized by Dr. H. A. Cole & Professor R. B. Clark. A full report is to be published in *Phil. Traver. R. Soc. B*.

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the sea each year (K.J. Whittle, MAFF Torry Research Station, Aberdeen). None of the following contributors who described the biological effects of oil defined what they meant by 'long term' and it was left to the penultimate speaker, Sir Hans Kornberg, FRS (Chairman Emeritus of the Royal Commission on Pollution) to quantify this term when he referred to the preceding papers as discussing "serious short term effects". Evidently the Royal Commission must regard long term as periods greater than the 10 to 20 years mentioned during the meeting as the time needed for recovery by some ecosystems after oil spills. In contrast, most of the contributors evidently thought that 10 to 20 years was a long time in comparison with the severe mortalities that ensue a few hours or days after major and minor spills (J.H. Vandermeulen, Bedford Institute of Oceanography, Dartmouth, N.S.).

It was suggested that repeated oil spills on one coast, such as in Brittany since 1967, might put off final recovery of affected communities to the next century (A.J. Southward, Marine Biological Association, Plymouth), but this was thought too pessimistic by the chairman (R.B. Clark, University of Newcastle) who noted that communities and ecosystems were remarkably resilient. This appears to be true for sea-birds, which suffer the most distressing casualties seen by the public, but which are mostly long-lived species undergoing heavy natural mortality each year, when hundreds of thousands die compared with tens of thousands killed by oil (G.M. Dunnett, University of Aberdeen). As confirmed in the discussion (W.R.P. Bourne, University of Aberdeen), some species can make up for heavy mortality in colonies by accelerated maturation of the juveniles.

Marine fishes suffer much heavier

natural mortalities than birds during their life history, and are also subjected to heavy pressure from fisheries. Fish populations, and catches by fishermen, undergo extensive natural fluctuations which, without the records of fishery statistics, might be ascribed to pollution rather than to natural changes or overfishing (R. Jones, DAFS Marine Laboratory, Aberdeen). Other marine populations, inleuding those on the seashore, undergo extensive natural fluctuations, and it may be difficult to detect pollution effects (J.R. Lewis, Wellcome Marine Laboratory, Robin Hood's Bay, Yorkshire). No general signs of oil-induced mortalities have been detected in commercial fish stocks (A.D. McIntyre, DAFS Marine Laboratory, Aberdeen), but tainting of the flesh may be evident after spills, and there is some suggestion of local inshore population changes in oysters and shrimp, related to chronic oil pollution. Other shellfish, especially Mytilus, are well known to be sensitive to low levels of petroleum hydrocarbons or other forms of sublethal environmental stress (B.L. Bayne, Institute for Marine Environmental Research, Plymouth), which can be detected as changes in metabolism and gonad development. It is therefore surprising that little general effect of natural oil seeps can be demonstrated in the Mytilus californianus community around Santa Barbara (Dale Straughan, Institute for Marine and Coastal Studies, University of Southern California, Los Angeles). Offshore petroleum operations in the Gulf of Mexico also appear to have had little effect on shell fisheries and the bottom fauna in general (J.M. Sharp, Gulf Universities Research Consortium, Houston), but signs of pollution-induced changes in the benthos are appearing close to a North Sea oil-rig (J.P. Hartley, Orielton Field

Centre, Pembroke).

Nevertheless, there is abundant experimental and circumstantial evidence that oil pollution is associated with histopathological lesions in fish and genetical abnormalities in pelagic eggs and larval stages (C.J. Sindermann, NOAA, Sandy Hook Laboratory, New Jersey). Such effects were seen in flatfish after the 'Amoco Cadiz' spill in Brittany (M. Conan, CNEXO, Brest), where long-lasting retention of oil in fine sediments may be delaying recovery of benthic animals and salt-marshes. It was thought that some saltmarshes, which were damaged by clean-up measures as well as oil, may take 20 years to recover. Comparable lengths of time have been suggested for tropical mangrove communities, which are very sensitive to oil damage (J. Baker, Orielton Field Centre, Pembroke). Both these types of community tend to be in places where oil spills are more likely.

The meeting demonstrated that there are long term chronic effects of oil, even if the evidence is partly circumstantial; but although damage from accidental spills can be severe and take many years to repair, the general effects are not as bad as once feared. It was felt that the tendency for committees to recommend further experiments on detection of effects of oil pollution should be resisted and replaced with ecological monitoring having well defined motives and objectives: there should be no hesitation in abandoning or reducing surveys where no ill effects were apparent. It is hoped that the recommendations of the Royal Commission will be acted upon by the Government, particularly those concerned with the prevention of oil spills, searches for clean-up techniques not dependent on chemicals and the setting-up of a central unit for coordinating action after spill.



#### 99 years ago

#### WEATHER FORECASTS

I have recently designed and patented "An improved floating vessel for automatically compressing air by the action of the waves of the sea, and also for the generation of electricity by the agency of this compressed air." This vessel is capable of being moored in 1000 fathoms and can be connected with the shore by means of an insulated electric cable. Such a vessel moored in the mid-Atlantic in the usual track of the cyclones which approach these islands from the west, would be of immense advantage to the Meteorological Office in determining the velocity of advance and direction taken by these cyclonic centres. I purpose exhibiting a model and drawings of the vessel at the Winter Electric Exhibition, to be held at the Westminster Aquarium next CHARLES W. HARDING month.

#### THE MAGNETIC STORM AND AURORA

The telegraphic system of this country has. since Friday morning last, been disturbed in a way that far exceeds anything of the kind that has ever happened before. Very powerful electric currents have been swaying backwards and forwards through the crust of the earth, taking all telegraphic circuits in their progress, and entirely stopping communication. Communication has been maintained only where it was possible to loop together two wires, so as to avoid the use of the earth altogether. The electric storm commenced on Thursday, but it reached its climax on Friday morning (November 17) between 10 and 11 a.m. The currents measured over 50 milliampères, which is five times greater than the ordinary working currents. They have repeated themselves at intervals ever since, but have scarcely attained such an intensity as on Friday morning.

Both the storm and the aurora seem to have extended to America; the Philadelphia correspondent of the *Times* telegraphs under date November 19:—

"The electrical storm which began to derange the telegraph wires on Friday last still continues, though with less intensity. It spread through Canada and the greater part of the United States, as far west as Utah. The electricians say that the disturbance was unlike any heretofore known, acting upon the wires in strong waves, which produced constant changes in the polarity of the current. A magnificent aurora appeared on Friday night and was visible at all points, except where clouds obscured it. Cold weather, with snow, accompanied the storm in many places."

Mr W.H.M. Christie, the Astronomer Royal, writes —

In the evening, as soon as it was dark, a brilliant aurora was seen, commencing with a bright glow of red light extending from the north and west beyond the zenith, interspersed with pale green phosphorescent light and streamers. At 6h. 4m. a very brilliant streak of greenish light about 20° long appeared in the east-north-east, and, rising slowly, passed nearly along a parallel of declination, a little above the moon, disappearing at 6h. 5m. 59s. in the west, about two minutes after it was first seen. The whole aurora had faded away by about 7h., but it burst out again at 11h. 45m., when an auroral arch, with brilliant streamers reaching nearly to the zenith, was seen from north-north-east to north-west.

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#### REVIEW ARTICLE

# λ Repressor and cro—components of an efficient molecular switch

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In a lysogen, most genes of phage  $\lambda$  are repressed; in response to a transient induction signal, they are efficiently switched on. The switch, which consists in part of a tripartite operator to which two regulatory proteins bind, depends not only on DNA-protein interactions, but also on effects transmitted from one DNA-bound protein to another.  $\lambda$  exemplifies a strategy that facilitates efficient switching between two physiological states in response to a transient signal.

THE genome of the temperate bacteriophage  $\lambda$  can exist in either of two states. Switching from one state (lysogeny) to the other (lytic growth) is induced with high efficiency by external agents such as UV light, and virtually all lysogens in a population can be induced synchronously by such treatments. The genes cI and cro, upon which lysogeny and lytic growth respectively depend, encode key components of the system. The protein product of each of these genes (repressor and cro) turns off the other gene. In a lysogen, repressor is made, but not cro; after the switch to the lytic cycle, cro but not repressor is synthesized (Fig. 1).

We have recently described the molecular mechanisms of action of repressor and cro¹. These two DNA-binding proteins recognize the same operator sites but have opposite physiological effects. The action of repressor is particularly elaborate: it regulates transcription both positively and negatively and, in doing so, engages in three different cooperative interactions. Why is the action of repressor so complex?

To explore this and related questions, we first review present knowledge of how  $\lambda$  repressor and cro function. We then consider studies, largely unpublished, of 434 and P22, two other temperate phages which have represssors, cro proteins and operators that differ in sequence from those of  $\lambda$ . We find that the general structures of the operators, the patterns of positive and negative control and the types of cooperativity shown by the repressor are similar in all three phages despite protein and DNA sequence differences and differences in detailed mechanism. We argue that these common features serve the same vital role for each of the three phages: they ensure stable lysogeny and allow efficient switching to lytic growth on induction. Thus, the lytic genes of the phage are very tightly repressed in the lysogenic state, yet they are efficiently and irreversibly switched on by a transient induction signal. Such a 'hairtrigger' response would be difficult to achieve with a repressor-operator system, such as that found in the lactose operon, that lacks the cooperative features of the  $\lambda$  system.

#### λ Repressor

Phage  $\lambda$  bears two operators to which repressor and cro bind. Here we consider mainly the right operator  $(O_R)$ , the primary locus of the lysogen-lytic growth switch. This operator consists of three sites, each of which can bind either repressor or cro, but not both simultaneously. These operator sites control two divergent promoters,  $P_R$  and  $P_{RM}$  (see Fig. 2). The activity of each of these promoters depends on which of the three operator sites are occupied by repressor and/or cro. For example, at  $O_R$  a

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prophage bears repressors bound mainly to sites  $O_{\rm R}1$  and  $O_{\rm R}2$ . In this state, repressor turns off transcription from  $P_{\rm R}$  and turns on transcription from  $P_{\rm RM}$ . As a consequence, the genes required for lytic growth (including cro) are repressed, while the gene required to maintain the lysogenic state (cI) is active. Repressor is a positive as well as a negative regulator: in its absence  $P_{\rm RM}$  functions very inefficiently. The experiments that elucidated the effect on gene transcription of repressor or crobound to each of the three sites in  $O_{\rm R}$  are described elsewhere  $^{1-7}$ .

Figure 3a shows a molecular model of the topography of repressors at  $O_R1$  and  $O_R2$  and RNA polymerase at  $P_{RM}$  in a  $\lambda$  lysogen. Three sets of protein-protein interactions are implicit in the figure: (1) dimer formation by repressor; (2) interaction between repressor dimers bound to  $O_R1$  and  $O_R2$ ; and (3) interaction between a repressor dimer bound at  $O_R2$  and an RNA polymerase molecule at  $P_{RM}$ . We shall consider these three different cooperative interactions in turn.

Formation of dimers.  $\lambda$  Repressor monomers are in equilibrium with dimers, but only dimers bind tightly to the operator<sup>8-10</sup>. One dimer occupies one operator site. Repressor monomers (236 amino acids) consist of two structural domains, each encompassing  $\sim 100$  amino acids, joined by a protease-sensitive connector<sup>11</sup>. The amino-terminal domains recognize the operator sites and the carboxy-terminal domains provide the most important contacts for dimer formation<sup>11,12</sup>. The binding in vitro of repressor to a single operator site has a sigmoidal dependence on repressor concentration, reflecting the requirement for dimer formation<sup>10,13</sup>.

Interactions between adjacent repressor dimers. Repressor dimers do not bind independently to the three sites in  $O_R$ ; rather

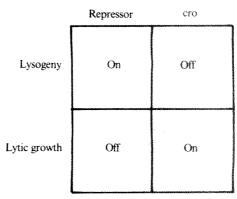


Fig. 1 Alternate physiological states of  $\lambda$ . In a lysogen, repressor is made but cro is not. During the early stages of lytic growth, cro protein but not repressor, is synthesized.

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they bind with positive cooperativity, and this interaction is crucial to repressor action<sup>4</sup>. We define the intrinsic affinity of each site for a repressor dimer as that observed in in vitro binding assays using DNA with a single functional site. Such experiments show that the independent affinities of  $O_R2$  and  $O_R3$  are about equal, and  $\sim 15$ -fold less than that of  $O_R1$ . However, when DNA containing all three operators, arranged as on the wild-type genome, is used, repressor binds to  $O_R1$  and  $O_R2$  cooperatively, but  $O_R3$  only binds repressor at much higher concentrations. The explanation for this lies in the interaction of repressors bound at  $O_R1$  and  $O_R2$ : repressor bound to  $O_R1$  raises the repressor-binding affinity of site  $O_R2$ , but not that of  $O_R3$ . The concentration of repressor in a lysogen is such that  $O_R1$  and  $O_R2$  are virtually saturated, but  $O_R3$  is largely unbound<sup>5,6</sup>.

Interaction between repressor dimers bound to sites  $O_R2$  and  $O_R3$  can be studied when  $O_R1$  is non-functional because of mutation. Repressor cannot then bind to  $O_R1$ , and a repressor bound to  $O_R2$  is free to interact with another at  $O_R3$ . As a result, the affinity of repressor for site  $O_R3$  is raised and that for  $O_R2$  is lowered relative to the wild type. Thus, a repressor dimer bound to  $O_R2$  interacts predominantly with another at  $O_R1$  (on a wild-type template) or with another at  $O_R3$  (on a mutant template in which  $O_R1$  has been destroyed). (We have called this phenomenon 'alternate pairwise cooperativity', and will return to its possible physiological significance later.) The  $O_R1-O_R2$  interaction predominates on a wild-type operator because  $O_R1$  is the tightest binding site.

We believe that these cooperative interactions are mediated by contacts between the carboxy-terminal domains or the domain connector regions of adjacently bound repressor dimers, because isolated amino-terminal domains bind to the three  $O_R$  sites specifically but non-cooperatively<sup>4</sup>. This result argues that the cooperativity is not due to a change in DNA structure induced by repressor binding. In addition, repressor at high concentrations in solution forms tetramers and higher oligomers<sup>8,10,14</sup>, perhaps using the same contacts as those involved in the cooperative binding to adjacent operator sites. We have suggested<sup>1,4</sup> that the inability of a repressor dimer bound to  $O_R 2$  to interact simultaneously with others at both  $O_R 1$  and  $O_R 3$  may be due to the limited flexibility and/or size of the connectors.

**Activation of P\_{\rm RM}.** We believe that repressor stimulates transcription from  $P_{\rm RM}$  by providing a favourable protein-protein contact with RNA polymerase <sup>1,6,7</sup>. The evidence for this view includes the following observations: (1) a repressor dimer bound to  $O_{\rm R}2$  stimulates transcription of  $P_{\rm RM} \sim 10$ -fold both in vivo and in vitro <sup>2,6,7</sup>. The primary role of  $O_{\rm R}1$  in this regard is to ensure that a repressor is bound to  $O_{\rm R}2$ . (2) Repressor and RNA polymerase bind to DNA cooperatively. For example, using DNase I as a probe, we found that formation of active ('open') complexes of RNA polymerase with  $P_{\rm RM}$  is enhanced if repressor is first bound to  $O_{\rm R}1$  and  $O_{\rm R}2$ . Conversely, when polymerase

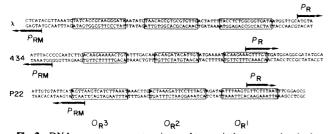


Fig. 2 DNA sequences, operator sites and transcription start-points in the right operators of  $\lambda$ , 434 and P22.  $O_R 1$ ,  $O_R 2$  and  $O_R 3$  are the sites recognized by the phage repressors. In the case of  $\lambda$ , we know that the three sites are also recognized by cro, and we presume that this is the case for 434 and P22 as well. Arrows mark the transcription start-points for the promotors  $P_R$  and  $P_{RM}$ . The operator-promoter regions are oriented so that in each case the repressor gene is transcribed to the left and the cro gene to the right. The  $\lambda$  operator sites consist of 17 bp; adjacent sites in  $O_R$  are separated by 6 and 7 bp. The 434 and P22 operator sites are 14 and 18 bp long, respectively. The 434 spacers consist of 8 and 9 bp, those of P22, 5 and 7 bp. Each operator site exhibits partial twofold rotational symmetry.

was bound to  $P_{\rm RM}$  (in the absence of repressor), the subsequent binding of repressor to  $O_{\rm R}2$  was similarly enhanced (A.J. and M.P., unpublished results). (In this experiment,  $O_{\rm R}1$ ,  $P_{\rm R}$  and  $O_{\rm R}3$  were made nonfunctional by mutation.) D. K. Hawley and W. R. McClure (personal communication) have reached similar conclusions and have further found that repressor stimulates formation of 'open' (but not 'closed') complexes of RNA polymerase with  $P_{\rm RM}$  (see refs 15–17). (3) Repressor bound at  $O_{\rm R}2$  is very close to (and may contact) polymerase at  $P_{\rm RM}$ . Figure 3b, which shows phosphates in close contact with repressor at  $O_{\rm R}2$  (and at  $O_{\rm R}1$ ) and polymerase at  $P_{\rm RM}$ , suggests that the proteins closely approach the same phosphate (and presumably, each other) between bases at positions -36 and -37 from the startpoint of  $P_{\rm RM}$  transcription (see Fig. 3 legend).

We believe that, as indicated in Fig. 3a, it is the aminoterminal domain of repressor that contacts RNA polymerase at  $P_{RM}$  and thus stimulates transcription from this promoter. Four lines of evidence suggest this. First, the purified amino-terminal domain of repressor activates transcription of  $P_{RM}$  in vitro 12 Second, RNA polymerase bound at  $P_{RM}$  enhances the binding of this amino-terminal domain to  $O_R2$  (A.J. and M.P., unpublished results). Third, a repressor fragment comprising the aminoterminal domain of repressor, when produced in large amounts, activates  $P_{RM}$  in vivo<sup>12</sup>. Fourth, a mutant of repressor has been isolated that represses  $P_{\rm R}$  but fails to stimulate  $P_{\rm RM}$  (as measured in vivo or in vitro<sup>69</sup>). The mutant repressor binds to  $O_R1$  and  $O_R2$  cooperatively with an affinity indistinguishable from that of wild-type. Plausibly this mutant maintains the structure(s) required for tight cooperative binding to DNA but lacks those necessary to interact with RNA polymerase. The mutation causes the change gly-arg at position 43 in the amino-terminal domain of repressor69

None of the experiments described above definitively excludes the possibility that repressor bound to  $O_R2$  stimulates  $P_{RM}$  by changing DNA structure. However, as repressor has little effect on the pitch of the DNA helix<sup>18</sup>, we find no reason to invoke an explanation involving changed DNA structure.

#### Induction and the role of cro

When Escherichia coli is treated with any of a variety of mutagens/carcinogens (as defined by the Ames test<sup>19</sup>), a set of genes known collectively as the 'SOS functions' is turned on (for reviews see refs 20, 21). One such gene encodes the recA protein which, when present in the low levels found in uninduced cells, catalyses genetic recombination. The recA protein is also a protease and, after an inducing treatment, it is produced at elevated levels and efficiently cleaves the  $\lambda$  repressor monomer at a specific site between the two domains<sup>22,23</sup>. (In vitro, this reaction requires cofactors presumably similar to those produced in vivo in response to inducing treatments.) The free amino-terminal repressor fragments produced by the cleavage cannot dimerize efficiently, and therefore cannot bind tightly to the operator<sup>11,12</sup>. The over-production and activation of recA (both probably required for extensive repressor cleavage) are transient phenomena, lasting for ~1 h after a typical inducing treatment (see refs 20, 24).

As the repressor in a lysogen is inactivated by cleavage,  $P_R$  is the first  $\lambda$  lytic promoter to be derepressed. This occurs because repressor binds less tightly to the sites in  $O_R$  than to those in  $O_L$ , the other major  $\lambda$  operator  $^{25,26}$ . The first new phage protein made is the product of the cro gene. The cro protein is a dimer (monomer length 66 amino acids)  $^{27,28}$  which, like the  $\lambda$  repressor, recognizes the three operator sites in  $O_R$ . DNA-bound cro is centred at each site identically to repressor, covering the same face of the helix and apparently contacting many of the same functional groups  $^{26}$ . Unlike the  $\lambda$  repressor, however, cro binds to the three  $O_R$  sites non-cooperatively, and its order of affinity to the three sites is reversed  $^{3,4}$ . That is, cro binds most tightly to  $O_R 3$ , and only at higher concentrations does it occupy  $O_R 2$  and  $O_R 1$ . Hence, after destruction of the repressor, cro is synthesized and first occupies  $O_R 3$ , as shown in Fig. 3e. In this configuration,  $P_{RM}$  is repressed and  $P_R$  is accessible to RNA

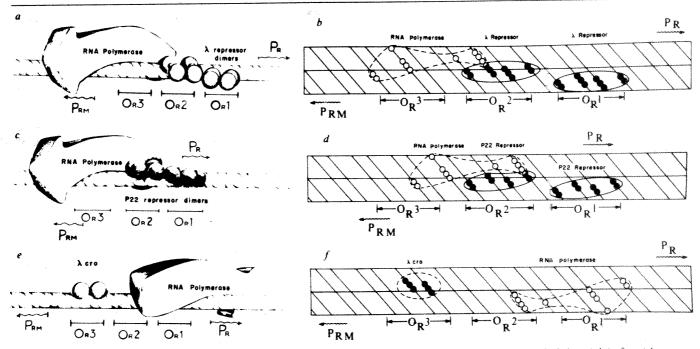


Fig. 3 Configuration of molecules bound to  $O_R/P_R$  in a  $\lambda$  lysogen (a, b), in a P22 lysogen (c, d), and during the early stages of  $\lambda$  lytic growth (e, f). a,  $\lambda$  lysogen: showing the topography of  $\lambda$  repressor dimers bound at  $O_R1$  and  $O_R2$ . This arrangement results in repression of  $P_R$  and activation of  $P_{RM}$ . b, Projection diagram showing the topography of  $\lambda$  repressor dimers bound at  $O_R1$  and  $O_R2$  and RNA polymerase bound to  $P_{RM}$ . The  $\lambda$   $O_R$  region of a is shown as a cylindrical projection (adopted from ref. 65) of B-form DNA with 10.4 bp per turn  $^{66}$ . ♠ Phosphates in close contact with bound  $\lambda$  repressor (see below). ⊖, phosphates believed to be in close contact with RNA polymerase bound at  $P_{RM}$  (see below). The solid and broken contour lines were drawn merely to group the phosphate contacts, not to imply the shapes of the DNA interaction sites. Note that the phosphate closely approached by both polymerase and repressor is marked by ⊕ c, P22 lysogen; dimers of P22 repressors are bound cooperatively to  $O_R1$  and  $O_R2$ , repressing  $P_R$  and activating  $P_{RM}$ . d, Topography of P22 repressors bound to  $O_R1$  and to  $O_R2$  and RNA polymerase bound to  $P_R$ . The symbols are as in b. Note that the relative positioning of P22 repressor at  $O_R2$  and polymerase at  $P_{RM}$  differs from that in the case of  $\lambda$  (b). e,  $\lambda$  lytic growth: after induction of a  $\lambda$  lysogen, cro proteins binds to  $O_R3$  thereby repressing  $P_RM$  and permitting  $P_R$  transcription. The precise positioning of these regulatory molecules on the DNA helix is based on the results of chemical probe experiments, some of which are summarized in e, e and e. Topography of  $\lambda$  cro bound to e0, and RNA polymerase bound to e1. Symbols are as above, cro makes four fewer phosphate contacts at each operator site than does repressor. The phosphates in close contact with a bound  $\lambda$  repressor e6.  $\mu$ 6 cro  $\mu$ 8 and P22 repressor  $\mu$ 9 were determined using the method of Maxam and Gilbert (pe

polymerase<sup>3,6</sup>. (When bound to  $O_R3$ , cro has no effect on  $P_R$  which, unlike  $P_{RM}$ , requires no auxiliary factor to function maximally.) Soon after lytic growth has commenced, as the concentration of cro increases, it turns down transcription from  $P_R$  by binding to  $O_R1$  and  $O_R2$ . This repression of early functions (including the production of cro) is essential for lytic growth<sup>29</sup>.

Two additional experiments (A.J., R. Maurer and M.P., unpublished results) emphasize the importance of the  $cro-O_R3$  interaction for induction. In the first, we studied  $\lambda$  lysogens bearing a plasmid which directs synthesis of cro under the control of the *lac* promoter. Synthesis of cro from the plasmid causes the lysogen to induce, even in the absence of repressor inactivation. This effect requires that the prophage carries an intact  $O_R3$ . We imagine that in this experiment, cro binds to  $O_R3$  and shuts off  $P_{RM}$ , and thus the production of repressor. Then, as the cells divide, repressor is diluted and induction occurs. The second experiment shows that binding of cro to  $O_R3$  is critical for efficient induction. We found that prophages mutated at  $O_R3$  in such a way that cro binds poorly, induce much less efficiently in response to UV irradiation than do wild-type prophages.

Once a phage genome reaches the state shown in Fig. 3e, we believe that it is committed to grow lytically. Hence, we view the two configurations of Fig. 3a and e as alternative states of the phage. Following induction, a prophage can be 'frozen' in the otherwise transient lytic state shown in Fig. 3e if it carries mutations which prevent expression of lytic genes other than  $cro^{30}$ . When the lysogenic bacterium bearing the mutant prophage is induced, it switches from the normal state where repressor but not cro is synthesized, to a new state in which cro but not repressor is synthesized (Fig. 3e). This new state, called 'anti-immune', like the normal lysogenic state, is inherited by subsequent generations.

#### Related phages

Which of the attributes of  $\lambda$  described above are vital to its life-cycle, and which are peculiar, perhaps accidental, to the  $\lambda$  case? To explore these questions, we now compare  $\lambda$  with two other phages, 434 and P22, to see which features are held in common. These other phages share with  $\lambda$  many basic biological properties; in particular, they all form UV-inducible lysogens. The genomes of the coliphages 434 and  $\lambda$  and of the Salmonella phage P22 are similarly organized, and share regions of homology<sup>31,32</sup>. Despite these similarities, the nucleotide sequences of the operators (Fig. 2) and the amino acid sequences of the repressors and cro proteins differ substantially. In particular, the repressor and cro of one phage do not recognize the operators of the others.

Nevertheless, we now argue that despite differences in operators, promoters and regulatory proteins, the following description holds for all three phages. The right-hand operator contains three repressor binding sites, two of which,  $O_R 1$  and  $O_R 2$ , are filled by repressor in a lysogen. This state depends on cooperative interactions between adjacent repressor dimers. Repressor monomers have two structural domains and are in equilibrium with dimers, the DNA-binding form. Repressor dimers occupying sites  $O_R 1$  and  $O_R 2$  turn off transcription from  $P_R$  and turn on that from  $P_{RM}$ . On UV induction, the repressor is cleaved and thereby inactivated, and the first protein synthesized from  $P_R$  is cro. The cro protein binds first to  $O_R 3$  to turn off repressor synthesis and later binds to  $O_R 1$  and  $O_R 2$  to decrease early lytic transcription.

The pictures we have developed for 434 and P22 are based on extensive experiments, in vivo and in vitro, similar to those performed for the analysis of  $\lambda$  (see ref. 1). In each case, plasmids that direct synthesis of large and variable amounts of the regulatory proteins were constructed by recombination in

vitro. The effects of repressor in vivo were analysed using these plasmids and other DNA molecules in which transcription of the lacZ gene was directed by a phage  $P_{\rm RM}$  or  $P_{\rm R}$ . The response of the wild-type and various mutant derivative promoters to different amounts of repressor was analysed. The interaction of the repressors, of cro proteins and of RNA polymerase with wild-type and mutant operators, as well as other properties of these proteins, were studied in vitro using purified components. By combining results from various such experiments, a coherent picture has emerged in each case.

Similarities in repressor structures. Like the  $\lambda$  repressor, the P22 and 434 repressors are folded into two structural domains which can be separated by proteases, including activated recA (refs 11, 33) and J. Anderson and M.P.; J. De Anda and R.S.; and R. Yocum and M.P., unpublished results). The aminoterminal fragments of 434 and P22 repressors bind specifically to their operators (J. Anderson and M.P.; J. De Anda, A.P. and R.S., unpublished results). Although the evidence is inconclusive, it seems that the P22 and 434 repressors must dimerize before binding to their operators  $^{9.34}$ .

The  $\lambda$ , 434 and P22 repressors share extensive homology in their carboxy-terminal regions (refs 35, 36; R. Yocum and M.P., unpublished results). When the sequences are aligned, ~30% of the amino acids are identical. The amino-terminal domains of the three phage repressors (and of the cro protein—see below) show considerably less, but still significant, homology (R.S. R. Yocum, A.P and R. Doolittle, unpublished results). Of particular interest is a conserved Ala-Gly near the middle of each repressor. The recA protease cleaves this bond in all three repressors (ref. 33 and R. Yocum and M.P., unpublished results). We surmise that these homologies reflect, at least in part, structures that are recognized and cleaved by the recA protease to effect phage induction (see ref. 33). We note that a cellular repressor of UV-inducible genes, the product of the lexA gene, is also cleaved by the recA protein; it shares substantial carboxy-terminal homology with the phage repressors and carries an Ala-Gly in or near the recA cleavage site (refs 37, 38 and R. Yocum, R. Brent and M.P., unpublished results).

Similarities in operator structure and repressor binding. Although the  $\lambda$ , 434 and P22  $O_R$ s differ in DNA sequence, they are arranged in a strikingly similar way: each consists of three homologous, partially symmetric repressor binding sites (Fig. 2 and refs 1, 39-41). As in the case of  $\lambda$ , repressor dimers of 434 and P22 do not bind independently to their three operator sites; rather they fill sites  $O_R1$  and  $O_R2$  cooperatively and coordinately<sup>41,42</sup>. In P22, this cooperativity presumably results from protein-protein interactions between carboxy-terminal sequences of adjacently bound repressor dimers, because as with  $\lambda$ , the isolated amino-terminal domains bind specifically but non-cooperatively to the  $O_R$  of P22 (J. De Anda, A.P. and R.S., unpublished results). As for  $\lambda$ , so with 434 and P22: if repressor cannot bind to  $O_R1$  because that site is mutant, the repressors fill sites  $O_R2$  and  $O_R3$  cooperatively<sup>41,42</sup>.

The intrinsic affinity of each phage repressor for its operator sites, together with the cooperative interactions, ensure that for all three phages, repressor binds with the affinity order  $O_R 1 \approx O_R 2 > O_R 3$ . In lysogens of  $\lambda$ , 434 or P22, the repressor level is such that almost all templates bear repressors at  $O_R 1$  and  $O_R 2$  and that  $O_R 3$  is free of repressor in a significant fraction of cells<sup>5,6,41,42</sup>.

Common patterns of gene control by repressor. Repressors bound to  $O_R1$  and  $O_R2$  in both P22 and 434 elicit the same transcriptional response as does  $\lambda$  repressor bound to the corresponding sites. That is, in each case, repressor turns off  $P_R$  and turns on  $P_{RM}$  (refs 6, 41, 42). However, this similarity is deceptive and, indeed puzzling, because the overlaps between the promoters and operators are different in the three cases. For example,  $O_R2$  in P22 is 11 base pairs (bp) closer to the transcriptional start of  $P_{RM}$  than is the  $O_R2$  of  $\lambda$  to its  $P_{RM}$  (Fig. 2). Nevertheless, in P22 as in  $\lambda$  (as well as in 434), repressor bound only to  $O_R2$  activates  $P_{RM}$ . At first glance, this is surprising because, as we have previously described for  $\lambda$ , very small

differences in the position of repressor can qualitatively change the effect on transcription. For example,  $\lambda$  repressor bound to  $O_R2$  alone (a condition achieved with appropriate operator mutants) turns off  $P_R$  and turns on  $P_{RM}$ , even though it is positioned exactly 1 bp closer to the former than the latter. We have argued that this 1-bp difference enables repressor to contact polymerase and aid its binding to one promoter  $(P_{RM})$ , excluding its binding to the other  $(P_R)$ . Nevertheless we believe that in spite of differences in promoter—operator overlap, a common mechanism of positive (as well as of negative) control obtains for  $\lambda$  and P22.

Positive control: In P22 as in  $\lambda$ , a protein-protein contact could be made between the amino-terminal domain of a repressor bound at  $O_R2$  and an RNA polymerase molecule bound at  $P_{RM}$ (see Fig. 3a, c). Moreover, the same region of RNA polymerase (shaded in Fig. 3) plausibly could be contacted in the two cases. This hypothesized site of contact is near a phosphate group which, according to the chemical probe experiments, is closely approached by both repressor at  $O_{\rm R}2$  and polymerase at  $P_{\rm RM}$ (compare Fig. 3b, d). In any case, the spatial relationship of polymerase and repressor differs in the two cases: for P22, repressor (at  $O_R2$ ) and RNA polymerase (at  $P_{RM}$ ) cover different faces of the same two turns of the DNA helix, whereas in  $\lambda$  the molecules are arranged more nearly in tandem. In the case of 434, the distance between  $O_R2$  and  $P_{RM}$  is very close to that for  $\lambda$ , and it is likely that the relationship of repressor at  $O_{\rm R}2$ and polymerase at  $P_{RM}$  is the same for  $\lambda$  and 434 (see Fig. 2). Negative control: Repressor bound to  $O_R1$  and  $O_R2$  excludes binding of RNA polymerase to  $P_R$ , a point readily visualized for the  $\lambda$  case by comparing Fig. 3b and f. 434 and P22 repressors also exclude binding of RNA polymerase to  $P_R$ , but in these cases, different sets of DNA contact points are masked 41,42 As expected from this model nuclease probe experiments (performed with  $\lambda$  and P22) show that binding of RNA polymerase to  $P_R$  and repressor to  $O_R1$  and  $O_R2$  are mutually exclusive  $^{26,42}$ . Moreover, in the case of  $\lambda$ , it has been explicitly shown that repressor bound either to  $O_R1$  or to  $O_R2$  represses

Common mechanism of cro action. Like  $\lambda$ , 434 and P22 also encode cro proteins. The 434 cro protein, roughly the same size as that of  $\lambda$ , has been isolated and partially characterized<sup>43</sup>. It binds specifically to (or near) the 434 operator. Strains bearing mutated 434 and P22 prophage can exist stably in the 'anti-immune' state (see above) in which repressor synthesis is turned off, but cro synthesis persists<sup>30,44</sup>. This suggests, by analogy with  $\lambda$ , that the cro proteins from these phages bind to  $O_R3$  more tightly than to the other two sites in  $O_R$ , and that cro bound to  $O_R3$  would repress  $P_{RM}$  but allow transcription of  $P_R$ .

An intriguing fact has recently emerged from a comparison of the  $\lambda$ , 434 and P22 cro and repressor amino acid sequences (R.S., R. Yocum, A.P. and R. Doolittle, unpublished results). When the cro sequences are aligned with those of the repressor amino-terminal domains, significant homologies are revealed. Some positions are conserved in all six proteins; others are occupied by amino acids of a similar chemical type. The homology between individual cro and repressor sequences is in some cases very strong, and in others, much weaker. For example, repressor and cro from 434 are strikingly similar in sequence (ref. 45 and R. Yocum, unpublished results) whereas λ repressor and  $\lambda$  cro show little obvious homology to one another, a point noted previously by us and others<sup>1,28,35</sup>. In particular, the 10amino acid  $\alpha$  helix of  $\lambda$  cro proposed by Anderson et al.<sup>46</sup> to fit into the major groove of operator DNA shows little homology with the corresponding region of  $\lambda$  repressor. However (as noted above), these regions (identified by homology) are very similar in cro and repressor of 434. It remains to be seen how these similarities and differences in amino-acid sequence contribute to the different order of binding, and hence physiological effects, of the three cro/repressor pairs. In any case, the similarities between the phage repressor and cro sequences suggest a common evolutionary origin for the six regulatory proteins.

### Cooperativity and phage induction

Here we argue that the features that are common to  $\lambda$ , 434 and P22, including the tripartite operator, the various forms of cooperativity and the action of cro, enable the phage to switch from one state (lysogeny) to the another (lytic growth) in response to a transient signal.

Repressor dimerization and cooperative binding of dimers to adjacent sites. Why are two sites,  $O_R1$  and  $O_R2$ , used to mediate the effects of repressor in a lysogen? Why does repressor dimerize and why do two dimers fill sites  $O_R1$  and  $O_R2$  cooperatively? At first glance, it might seem that a stable dimer binding tightly to a single site  $(O_R2)$  would suffice. We believe that the dimerization and subsequent cooperative repressor binding to two adjacent sites ensures tight repression but maintains the system in a state poised to respond dramatically to a rather modest decrease in repressor concentration.

This conclusion is illustrated in part by Fig. 4, which indicates that  $P_R$  repression is a steep function of repressor concentration. This curve was calculated from data describing the binding of repressor to various wild-type and mutant templates in vitro (see Table 1 and Fig. 4 legend). Our measurements and calculations<sup>47</sup> indicate that in a lysogen, the degree of repression of  $P_R$  is at least 99% (see Fig. 4 legend). According to the curve, inactivation of 90% of the repressor would suffice to derepress  $P_R$  to  $\sim 50\%$  of its maximal level. This response is in striking contrast to that produced by a system involving stably oligomeric repressor binding to a single operator site (as exemplified by lac) which maintains an equivalent amount of

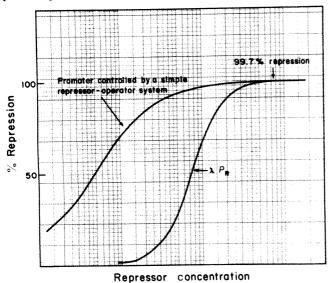


Fig. 4 Theoretical repression curves for  $\lambda P_R$  and a simple repressoroperator system such as lac. The curves show the extent of repression as a function of total repressor concentration. They were calculated as follows.  $\lambda P_R$ : at each total repressor concentration, the concentration of repressor dimers was calculated from the known equilibrium dissociation constant  $(K_D = 2 \times 10^{-8} M)^{10}$ . The distribution of repressor dimers among each of the eight possible states of the operator (Table 1) was calculated from a knowledge of the relative energy of each state using standard methods of statistical thermodynamics<sup>47</sup>. The energies of each of the eight states of Table 1 were deduced from DNA binding studies carried out in vitro<sup>4</sup>. A repressor dimer bound either to  $O_R1$ ,  $O_R2$  or to both sites, will repress  $P_1$ For any repressor dimer concentration, therefore, the fraction of  $O_R$  templates with  $P_R$  repressed is given as the sum of states 1, 2, 4, 5, 6 and 7 from Table 1. Simple repressor-operator system: for comparison we show a theoretical repression curve for the case of a protein (of a stable oligomeric form) binding to a single DNA site (that is, lac repressor binding to its operator). The curve was generated from the simple relation,  $OR/O_T$  $1/(1+K_D/R_T)$  where  $OR/O_T$  is the fraction of operators occupied by a repressor,  $R_T$  being the total repressor concentration, and  $K_D$  is the dissociation constant describing the reaction RO = R + O (ref. 68). The two curves in the figure were aligned at the 99.7% repression point. We believe this to be a minimum estimate of the extent of repression found in a  $\lambda$ lysogen. This value was derived from the knowledge<sup>5</sup> that in a lysogen,  $O_R3$ is  $\sim 20\%$  repressed. According to our calculations, when  $O_R3$  is 20% repressed,  $P_R$  is at least 99.7% repressed. We have no direct way of measuring accurately the extent of repression actually found in a lysogen. However, we know that the extent of repression of the lac operon in vivo is ~99.9%

Table 1 Eight possible configurations of repressor at  $O_R$ 

	Configuration			Free-energy	Total free energy
State	$O_{R}3$	$O_R2$	$O_R1$	contributions	(kcal mol <sup>-1</sup> )
0				(reference state)	0
1			+	$\Delta G_1$	-11.6
2		+		$\Delta G_2$	-10.1
3	+			$\Delta G_3$	-10.1
4		+	+	$\Delta G_1 + \Delta G_2 + \Delta G_{12}$	-23.7
5	+		+	$\Delta G_1 + \Delta G_3$	-21.7
6	+	+		$\Delta G_2 + \Delta G_3 + \Delta G_{23}$	-22.2
7	+	+	+	$\Delta G_1 + \Delta G_2 + \Delta G_3 + \Delta G_3$	$G_{12} = -33.8$

+ Indicates that the corresponding site is occupied by a repressor dimer. The free energies of each state (relative to the reference state) are listed.  $\Delta G_1$ ,  $\Delta G_2$  and  $\Delta G_3$  are the intrinsic interaction energies of repressor dimers binding to each of the three sites.  $\Delta G_{12}$  is the net interaction energy supplied by the cooperative interaction between a repressor dimer at  $O_R1$  and one at  $O_R2$ .  $\Delta G_{23}$  is similarly defined. These values, measured in 'physiological conditions' (200 mM KCl, 37 °C) are  $\Delta G_1 = -11.6$  kcal mol<sup>-1</sup>,  $\Delta G_2 = \Delta G_3 = -10.1$  kcal mol<sup>-1</sup>,  $\Delta G_{12} = \Delta G_{23} = -2.0$  kcal mol<sup>-1</sup> (ref. 4) (see Fig. 4 legend).

repression (see Fig. 4). In this case, 50% induction requires inactivation of a much larger fraction of repressor (>99%), and inactivation of 90% of the repressor would result in only  $\sim 3\%$  derepression and thus induction. There is evidence that inactivation of 90% of the  $\lambda$  repressor in a lysogen produces highly efficient induction<sup>24</sup>. Both repressor dimerization and cooperative binding to  $O_R1$  and  $O_R2$  contribute to the steep slope of Fig. 4 (ref. 47).

 $P_{\rm RM}$  activation and the action of cro protein. Once  $P_{\rm R}$  of a prophage is derepressed, why does the phage not simply reestablish repression as the inducing signal decays? We believe that the dependence of  $P_{\rm RM}$  activity on bound repressor and the action of cro prevent the re-establishment of lysogeny and promote lytic growth. As repressor present in a lysogen is inactivated, it vacates  $O_{\rm R}$ . A template lacking a repressor at  $O_{\rm R}2$  no longer directs synthesis of repressor efficiently, and thus the rate of repressor synthesis declines. At the same time, cro is synthesized, and by binding first to  $O_{\rm R}3$ , turns off repressor synthesis and promotes lytic growth.

Thus in summary the three related phages,  $\lambda$ , 434 and P22, encode specific repressor and cro proteins that interact in each case with a key regulatory sequence, termed the right operator. We find that despite detailed differences in mechanism, in each case repressor in a lysogen binds cooperatively to the tripartite operator, turning off lytic genes and stimulating transcription of its own gene. We have argued that a common molecular mechanism of positive and negative control obtains in each case, despite differences in operator-promoter overlap. Moreover, we argue that the common features of repressor action, including in each case its cooperative interaction with a tripartite operator, have been maintained to ensure the switch from lysogenic to lytic growth. Thus, in a lysogen, repressor is bound predominantly to  $O_R1$  and to  $O_R2$ , thereby repressing  $P_R$  and activating  $P_{RM}$ ; to a lesser extent repressor is bound to  $O_R3$ , thereby turning down repressor synthesis.

The lysogenic state is stably inherited; lysogens spontaneously induce only about once per  $10^5$  generations. On exposure of lysogens to inducer, cleavage of repressor commences. A 5-10-fold drop in repressor level triggers a dramatic change of state as  $O_{\rm R}1$  and  $O_{\rm R}2$  are vacated: because repressor synthesis depends on autogenous stimulation, its rate of synthesis decays rapidly. Moreover, induction of  $P_{\rm R}$  results in synthesis of cro, a protein required for lytic growth, which binds to  $O_{\rm R}3$ , turns off  $P_{\rm RM}$ , and thereby completes the switch to lytic growth. The various forms of protein-protein interaction involving repressor ensure stability of the lysogen but permit  $P_{\rm R}$  to begin functioning in response to a moderate decrease in repressor level.

#### **Further considerations**

**Role and positioning of O\_R 3.** We have explained that the two sites  $O_R 1$  and  $O_R 2$  and their interaction serve an important

biological function that could not be met by a single repressor binding site. We have also described a role for  $O_R3$ , that is, turning off repressor synthesis by ero and thus promoting lytic growth. We now suggest two additional functions for  $O_R3$ .

First, repressor can also bind to  $O_R3$  and turn off transcription of its own gene. We have found that in lysogens,  $O_R3$  of  $\lambda$  is  $\sim 20\%$  occupied (and  $O_R3$  of P22 slightly more), whereas sites  $O_R1$  and  $O_R2$  in both  $\lambda$  and P22 lysogens are almost completely filled 5.6,41,42. The negative autogenous control mediated by repressor binding to  $O_R3$  should help to maintain constant repressor concentrations, but apparently the effect is not dramatic. For example, we have found that a fivefold increase in  $\lambda$  repressor concentration decreases the rate of repressor synthesis only by  $\sim 20\%$  (ref. 5); we do not know whether this response is physiologically important. Perhaps slight decreases in repressor levels that otherwise might trigger induction are compensated for by increased repressor synthesis as  $O_R3$  is vacated. It is possible that negative autogenous control in 434 is more extensive than in the other two phages (see ref. 9), but our understanding of the situation is incomplete.

Second, as we have mentioned elsewhere<sup>1</sup>, we belive that  $O_R3$  helps to prevent the accumulation of  $\lambda$  virulent mutants—variants that grow lytically (and only lytically) on lysogenic as well as on non-lysogenic cells, and thereby destroy the lysogenic system. A requirement for virulence is that transcription from  $P_R$  proceed efficiently in the presence of repressor at the concentration found in a lysogen. In general<sup>48</sup>, two sites in  $O_R$  must be mutant, either one in  $O_R1$  and another in  $O_R2$  or one in  $O_R1$  and another in  $O_R3$ .

We understand this requirement for two mutations to be as follows. (1) If a phage acquires a mutation in  $O_R 1$  alone,  $P_R$  will nevertheless be repressed because the cooperative interaction between repressor dimers at  $O_R2$  and  $O_R3$  ensures that  $O_R2$  will be occupied. Addition of a second mutation in  $O_R2$  or in  $O_R3$  is necessary for  $P_R$  to escape repression. This hypothesized role of  $O_{\rm R}3$  (but not the first two described above) requires that  $O_{\rm R}3$  be positioned close to  $O_R2$  so that the necessary cooperative interaction can occur. (2) If a phage acquires a mutation in  $O_R 2$ , the high intrinsic affinity of O<sub>R</sub>1 for repressor nevertheless ensures that  $P_R$  will be repressed, and an additional mutation in  $O_{\rm R}1$  is required. Thus, the pattern of intrinsic affinities and alternate cooperative repressor binding to the three sites imposes the requirement that two mutations, rather than one, must be accumulated for  $O_R$  to escape repression. Furthermore, phage that have acquired one operator mutation (in either  $O_R 1$ or  $O_R 2$ ) cannot lysogenize (because occupancy of  $O_R 1$  and  $O_R 2$ , but not of  $O_R3$ , is required for lysogeny) and such phage are thereby presumably at a selective disadvantage compared with wild-type.

Efficiencies of  $\lambda$  and lac repressor. The affinity  $(K_{\rm D})$  of a  $\lambda$  repressor dimer for  $O_{\rm R}1$  on a wild-type template in 'physiological conditions' (37 °C, 0.2 M KCl) is  $3 \times 10^{-9}$  M (refs 4, 10, 13). The other, well-studied repressor, the lac repressor, binds to its operator much more tightly in these conditions  $(K_{\rm D}=10^{-12}\,{\rm M})^{49}$ . Nevertheless, as argued in the text, we believe that  $\lambda$  repressor in a lysogen turns off  $P_{\rm R}$  about as efficiently as lac repressor turns off the lac promoter (repression  $\geq 99.7\%$ ).

The  $\lambda$  repressor achieves a similar degree of repression despite a lower binding constant because of the following factors. First, the  $\lambda$  repressor is present at 5-10-fold higher concentration in a cell than is lac repressor. (There are ~200 monomers of  $\lambda$  repressor  $(10^{-7} \, \mathrm{M})$  in a lysogen<sup>8,9,24,50</sup> and the  $K_D$  describing dimer formation is  $2 \times 10^{-8} \, \mathrm{M}$  (ref. 10), whereas the concentration of lac repressor stable species (tetramer) is 10-20 molecules per cell<sup>51,52</sup>.) Second, the affinity of lac repressor for non-operator DNA may decrease its effective concentration in E. coli relative to  $\lambda$  repressor. For lac repressor, the ratio of affinities for nonspecific to operator DNA is  $\sim 10^{-8}$  (ref. 49). The binding constant of lac repressor for random 20-bp non-operator sequences  $(10^{-4} \, \mathrm{M})$ , taken with the

total concentration of such sequences in E. coli  $(10^{-2} \,\mathrm{M})$ . suggests that an appreciable fraction of repressor is sequestered on nonspecific DNA<sup>49,53</sup>. Linn and Riggs<sup>49</sup> have argued that this effectively weakens the binding constant of lac repressor by two orders of magnitude. We know that for  $\lambda$  repressor, the ratio of nonspecific to specific DNA binding is  $\sim 10^{-8} \,\mathrm{M}$  at low salt and low temperature 10. Assuming that this ratio is the same in 'physiological conditions' (see above), we estimate that little or none of the  $\lambda$  repressor in a lysogen is bound to non-operator DNA. Third, a consequence of repressor dimerization and of cooperative binding of repressors to  $O_R1$  and  $O_R2$ (see Fig. 4) is that 99.7% repression is reached when the concentration of repressor is ~12-fold above that needed to fill half the sites (that is, the apparent  $K_D$ ), and this is the amount of repressor found in a lysogen. In the lac case, this degree of repression is achieved only when the concentration of repressor exceeds the  $K_D$  300-fold.

**Positive control.** We have described a particular mechanism whereby the phage repressors mediate positive control of transcription in  $\lambda$ . Note that we have paid little attention to an alternative mechanism, which we now discuss.

It is conceivable that RNA polymerase bound to the strong promoter,  $P_{\rm R}$ , would exclude binding of polymerase to the weak promoter,  $P_{\rm RM}$ , and that the role of repressor in positive control is to prevent binding of polymerase to  $P_{\rm R}$ .

This model deserves serious consideration for  $\lambda$ , 434 and P22 because in each case the two promoters,  $P_R$  and  $P_{RM}$ , are adjacent. A series of experiments argues strongly that in  $\lambda$ , this model is excluded. Polymerase bound at  $\lambda$   $P_R$  has little inhibitory effect on binding of polymerase to  $P_{RM}$ , and removing  $P_R$  does not activate  $P_{RM}^{}$ . In the case of P22 and 434,  $P_R$  and  $P_{RM}^{}$  are significantly closer than in  $\lambda$ , and although we have no definitive evidence, we believe it likely that in each case polymerase bound to one inhibits binding of polymerase to the other  $^{41,42}$ . Nevertheless, deletion of  $P_R^{}$  of P22 does not activate its  $P_{RM}^{}$ : rather, as in  $\lambda$ , repressor must be bound to the DNA for  $P_R^{}$  stimulation. Thus, in  $\lambda$ , the repressor provides an essential contact with polymerase at  $P_{RM}^{}$ , whereas it is likely that in P22 and 434, the repressor both excludes a competing polymerase at  $P_{RM}^{}$ .

Analogy with haemoglobin. A haemoglobin tetramer bearing a bound oxygen has a higher affinity for oxygen than does a haemoglobin tetramer bearing no oxygen<sup>54</sup>. As a result, a small change in oxygen pressure can cause a dramatic change in oxygen binding to haemoglobin. The interaction of the  $\lambda$  operators with  $\lambda$  repressor and of haemoglobin with oxygen are formally analogous. As shown in Table 1, the energy of interaction of repressor for  $O_R1$  alone is -11.6 kcal mol<sup>-1</sup>, and that for  $O_R2$  alone is -10.1 kcal mol<sup>-1</sup>. The net interaction energy between adjacent repressor molecules is -2 kcal mol<sup>-1</sup>, which means that the effective energy of binding to the second site on a wild-type template is -12.1 kcal mol<sup>-1</sup>. Thus, an operator containing a bound repressor has a higher affinity for a second repressor than does an operator bearing no repressor. As a consequence, small changes in repressor concentration can have a dramatic effect on repressor binding to the operator (see Fig. 4). An important difference in the analyses of  $\lambda$  and haemoglobin is that in the latter case, one must measure the total amount of ligand (oxygen) bound. In contrast, for  $\lambda$ , we can quantitate binding of the ligand (repressor) to each of the three sites in the operator. This greatly simplifies analysis of mechanism (see ref. 47).

Other cases. Interactions between adjacent DNA-bound proteins probably also play an important part in both prokaryotic (see, for example, refs 70-73) and in eukaryotic gene regulation. For example, transcription of the SV40 early genes (including that which encodes T antigen) is repressed by the T antigen<sup>55-59</sup>. This protein binds to three sites near the early transcription start-point. Binding of the purified protein to the middle site is enhanced by previous binding to the first<sup>60</sup>. There is evidence<sup>60</sup> that this cooperative binding is mediated by pro-

tein-protein interactions between adjacent T-antigen molecules.

In another case, transcription of the 5S gene of Xenopus in partially fractionated oocyte extracts is stimulated by a protein that binds specifically to a site within the gene<sup>61</sup>. One interpretation of the experiments of Sakonju et al.<sup>61,62</sup> is that the function of the stimulatory factor is to promote binding of RNA polymerase to the (relatively nonspecific) adjacent DNA sequence.

In prokaryotes, as well as eukaryotes, transient signals can induce epigenetic changes which are then stably inherited<sup>63,6</sup> The phage cases we have analysed demonstrate one molecular mechanism for ensuring such a response. More generally, it is

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plausible that at one or more stages during the development of higher organisms, differences between daughter cells depend on the asymmetric distribution of cytoplasmic determinants formerly present in the parental cell. Our analysis of the  $\lambda$  case shows how a relatively small (<10-fold) change in such factors can cause a drastic epigenetic change.

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## ARTICLES

## Oscillatory zoning: a pathological case of crystal growth

## Claude J. Allègre, Ariel Provost & Claude Jaupart

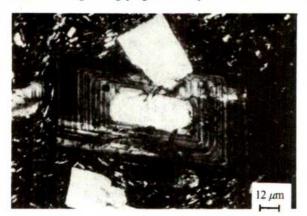
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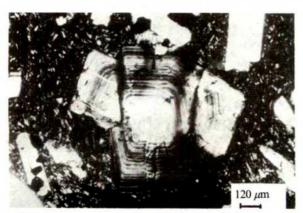
A new theory of oscillatory zoning in naturally grown plagioclase crystals is presented. This describes explicitly the coupling between the interface kinetics and the diffusion of chemical species in the melt. The crystal growth rate R responds with a finite delay time to concentration changes at the interface. Thus the growth rate cannot be simply some function of the supersaturation. Oscillatory zoning occurs when growth rate is low. This theory accounts for the absence or extreme rareness of oscillatory zoned plagioclase crystals in laboratory growth experiments.

PLAGIOCLASES are usually compositionally, and thus optically, zoned. On large scale irregular and abrupt changes in composition (stepped zoning) are often superimposed almost periodic variations (oscillatory zoning). This 'mineral stratigraphy' has long interested petrologists because it offers a potentially useful record of environment changes during crystallization<sup>1-7</sup>. It is generally accepted that stepped zoning is created by physicochemical variations in the magma whereas oscillatory zoning is a local phenomenon due to crystal growth processes. However, no quantitative analytical theory has been developed, and the available theories do not explain why the plagioclase–melt system is one of the few natural systems to exhibit this phenomenon.

Zoning patterns in plagioclase crystals have been described elsewhere <sup>1-4,8-12</sup>. Typical oscillatory zoning characteristics are illustrated in Fig. 1: oscillations occur as groups separated by abrupt changes. The sequence from inner to outer groups generally displays a trend towards greater albite content. The oscillation width is almost constant for a given group but may vary from group to group in the 10–30 µm range. Each concentration peak has a characteristic shape, usually a smooth Ab increasing flank followed by a steep Ab decreasing flank, although there may be no asymmetry in some cases<sup>7</sup>.

We do not discuss the arguments based on constitutional supersaturation which favour an 'internal' origin for oscillatory zoning (see, for example, refs 4, 6 and 7) but from an observational point of view, it is remarkable that no inter-crystal correlation of oscillation bands has been reported<sup>13</sup>. Several authors<sup>6,14,15</sup> have demonstrated the existence of concentration gradients before growing plagioclase crystals and stressed the





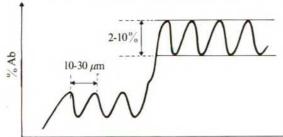


Fig. 1 a, Two examples of oscillatory zoning in plagioclase crystals. Samples are basaltic andesites from the Colima volcano (Mexico) (photographs provided by C. Bollinger). b, Cross-section of an oscillatory zoned portion of a plagioclase crystal: schematic plot of composition versus distance (from refs 6, 7).

Distance from core

fundamental role of chemical diffusion. There is clear evidence for a near-crystal layer enriched in elements with solid-liquid partition coefficients smaller than 1 (Mg, Fe, Si, Na)<sup>6</sup>.

The phenomena which occur during crystallization from a liquid can be broadly classified as interactions between three important variables: (1) the crystallographic features of the growing crystal and the associated effects on the interface kinetics, (2) the temperature distribution in the liquid, (3) the distribution of elements in the liquid.

In the present context, heat diffusion is much more efficient than chemical diffusion and temperature is not a limiting variable for the low growth rates considered. The competitive character of interface kinetics and chemical diffusion alone does not explain the phenomenon of oscillatory zoning. Consider a newly nucleated crystal: there is no diffusion layer in front of the interface, and growth begins with a fast increasing Ab concentration. Eventually, a steady-state regime should be reached with equal growth and diffusion rates. Kinetic effects may explain why this may not occur.

#### Equations for plagioclase growth

We now consider the equations which govern the interface kinetics—chemical diffusion interaction. Assuming a flat interface, we consider a coordinate system which moves with the crystal surface, and restrict the discussion to one-dimension, two-component fickian diffusion for simplicity. Strictly, our single independent component is that eigen-vector of the diffusion coefficient matrix which is associated with its lowest eigenvalue D, but as it is comparatively Si-rich and Al-poor, it will be labelled 'albite' for clarity. Albite concentration in the melt at a distance x from the interface, C(x, t), is governed by:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} + R \frac{\partial C}{\partial x}$$
 (1)

where R(t) is the crystal growth rate. If slower solid diffusion is neglected, mass balance at the interface reads:

$$R(C_0 - C_s) = -D \frac{\partial C}{\partial x}\Big|_{x=0}$$
 (2)

where  $C_0(t) = C(0, t)$  and  $C_s(t)$  stand for liquid and solid Ab concentrations at the interface. The second boundary condition is a 'far away fixed concentration' condition:

$$C(x_1, t) = C_{\rm m} \tag{3}$$

where  $x_1$  is the distance at which mean magma Ab concentration  $C_m$  is maintained. For simplicity,  $x_1$  is taken as infinite throughout.

For given initial conditions, two more boundary conditions must be specified. In the limiting case of instantaneous interface kinetics (when growth is controlled by diffusion in the vicinity of the interface), these are the thermostatic equations  $C_0 = C_L$  and  $C_s = C_S$ , where  $C_L$  and  $C_S$  are the liquidus and solidus Ab concentrations. In the other limiting case of instantaneous diffusion (interface kinetics controlled growth), equations (1) and (2) degenerate and  $C_S$  and R are functions of  $C_0 = C_m$ . In the more intricate intermediate situations, the two required conditions must link  $C_S$  and R to  $C_0$  and t, but there are few constraints from experiments or theory (see refs 16 and 17 for discussions). There is, however, some experimental evidence that the partition coefficient  $K = C_S/C_0$  remains approximately constant even when R changes by an order of magnitude 18.

Most theories explicitly or implicitly state that  $C_s$  and R are single-valued functions of  $C_0$ , as they are in the limiting case of instantaneous diffusion (well-stirred solutions or melts). But, complicated though these functions may be, equations (1) (2) and (3) cannot produce oscillations, because of the presence of only a first-order time derivative (this is at variance with mechanical or electrical oscillators for which a second-order or two coupled first-order differential equations are involved).

Bottinga et al.<sup>6</sup> and Sibley et al<sup>7</sup> suggest a hysteresis behaviour for R. Oscillatory zoning requires a jump from very slow growth

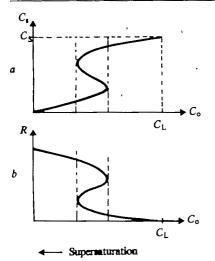


Fig. 2 According to Haase et al.  $^{21}$ , the albite content  $C_s(a)$  of a growing plagioclase crystal and its growth rate R(b) may be triple-valued functions of  $C_0$  (the albite content at interface) over a finite interval of supersaturations. This figure is not drawn to scale.

to no growth, which in turn has to wait for a new nucleation step. But their discussions offer no clear explanation for such a jump and rely on an 'interface diffuseness' concept<sup>19</sup> which seems questionable<sup>20</sup>.

The kinetic theory proposed by Haase et al.  $^{21}$  considers  $C_s$  and R as functions of  $C_0$  and  $C_s$ , then predicts that  $C_s$  and thus R are tripled-valued functions of  $C_0$  on a certain interval of  $C_0$  values (Fig. 2). They argue that  $C_s$  and R oscillate between the lowest and the uppermost branches (the middle one being clearly unstable with respect to chemical diffusion). But they do not explain why R and  $C_s$  stay on a given branch as long as possible, nor does the theory describe the necessary up and down jumps. This points to a failure of the kinetic model which may be modified either by considering a discontinuity (analogous to phase changes or shock waves) or by adding a dynamic term (such as finite response time for one variable). Only the latter may lead to an oscillatory behaviour.

The important question is therefore: does the growth rate respond immediately to the changing composition? The advance of a solid-liquid interface is known, both theoretically  $^{22,23}$  and experimentally  $^{24}$ , to depend critically on the detailed structure of the interface on the molecular scale. As site formation is a probabilistic process, there will be a delay between a change in  $C_0$  and a change in R. A plausible equation for R is thus:

$$\tau \frac{\mathrm{d}R}{\mathrm{d}t} + R = \Re(C_0) \tag{4}$$

where  $\tau$ , the characteristic time for structural rearrangement, is taken as constant for simplicity. Samoylovich<sup>25</sup> proposed a similar equation for the advance of a crystallization front with prescribed thermal gradients at the boundaries of the crystallizing region.

If  $\tau$  is zero or steady-state obtains, equation (4) reduces to  $R = \Re(C_0)$ , that is the growth rate is some function of the supersaturation which may be determined by laboratory experiments. According to what is known of nucleation and growth mechanisms, the positive function  $\Re(C_0)$  must vanish for  $C_0 = C_L$  and increase with decreasing  $C_0$  (at least for moderate supersaturations). Some indication of its magnitude and sensitivity to concentrations and temperature is provided by data from ref. 15. The essence of our analysis lies in equation (4) where we suggest that R should be considered as the solution of a differential rather than algebraic equation. Although this may represent a reasonable approach to growth mechanisms, it should be regarded as phenomenological. We show next that the proposed coupling between R and  $C_0$  results in an oscillatory behaviour consistent with observations.

### A quantitative model

Before dealing with equations (1) and (4), we describe qualitatively what happens during a typical episode of crystal growth. At time t=0, growth starts in a regime controlled by the

interface kinetics. Initially the concentration profile in the melt phase is flat and  $C_0$ , the concentration at the interface, is equal to  $C_{\rm m}$ . As growth proceeds, a concentration boundary layer develops ahead of the crystal and growth becomes progressively diffusion controlled. As time increases,  $C_0$  tends to  $C_L$  and the concentration profile tends asymptotically to that described by the boundary condition  $C_0 = C_L$  (ref. 26, p. 146). Therefore, the boundary layer thickness  $\delta$  behaves asymptotically as  $\sqrt{t}$ , and R behaves as  $1/\sqrt{t}$ . In this limit, the function  $\Re(C_0)$  in equation (4) may be approximated by an equation such as:

$$\Re(C_0) = Q(C_L - C_0)^* \tag{5}$$

This general form of  $C_0$  and R is sketched in Fig. 3. Throughout this evolution, the concentration boundary layer thickness  $\delta$  increases as the crystal evolves towards higher and higher albite concentrations.

The situation is more complex for an oscillatory growing crystal. However, the observations show that the oscillations are of small amplitude and are superimposed on a regular evolution which follows along the lines described above. This will lead us to write the general solution as the sum of a regular slowly-varying solution and a perturbation of small amplitude. A remarkable aspect of the governing equations is that a qualitative knowledge of the regular solution enables us to predict the behaviour of the perturbations. We integrate equation (1) and obtain:

$$\int_{0}^{\infty} \frac{\partial C}{\partial t} dx = -D \frac{\partial C}{\partial x} \Big|_{x=0} + R(C_{m} - C_{0})$$
 (6)

We define a diffusion width  $\xi(t)$ , which is a measure of the thickness of the concentration boundary layer, as:

$$\int_{0}^{\infty} (C - C_{\mathbf{m}}) \, \mathrm{d}x = \xi (C_{0} - C_{\mathbf{m}}) \tag{7}$$

Using equation (7) and boundary conditions (2), equation (6) may be written as:

$$\frac{\mathrm{d}}{\mathrm{d}t} \left[ \xi (C_0 - C_{\mathbf{m}}) \right] = R(C_{\mathbf{m}} - KC_0) \tag{8}$$

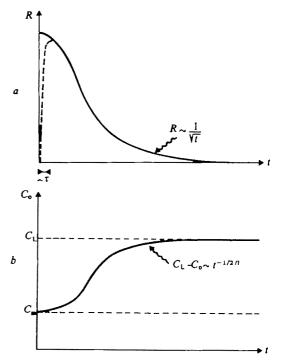


Fig. 3 Plots of the growth rate R(a) and the albite content of the melt at the interface  $C_0(b)$  against time for a typical episode of crystal growth. When the conditions for oscillatory zoning are not fulfilled, it makes little difference whether there is a delayed response of the growth rate (dashed line in a) or not (solid line). n in b is the same as in equation (5).

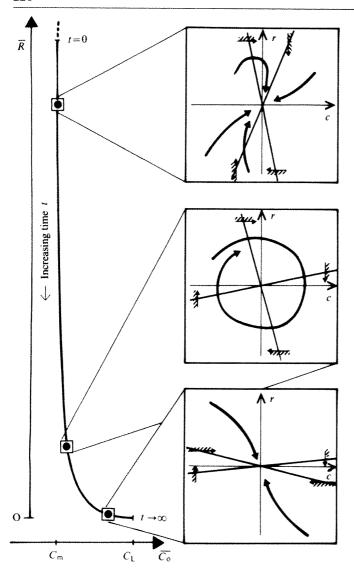


Fig. 4 Phase-plane representation of the solution  $\{R, C_0\}$ . The heavy curve on the left represents the evolution of the quasistationary regular solution  $\{\bar{R}, \overline{C_0}\}$ . Superimposed on that solution is a perturbation  $\{r, c\}$  whose evolution with respect to the regular solution is shown in the three insets at three different stages of growth. In the insets, straight lines represent the isoclines dc/dt = 0 (equation (12)) and dr/dt = 0 (equation (13)), and arrows represent tangents to the evolution curves. We predict the perturbation  $\{r, c\}$  to be rapidly and monotonically flattened out at both the beginning (upper inset) and the end (lower inset), but to exhibit damped oscillations (middle inset) over a finite time interval.

We now write the full solution  $\{R, C_0\}$  as the sum of a slowly-varying regular solution  $\{\overline{R}, \overline{C_0}\}$  and a perturbation of small amplitude  $\{r, c\}$ :

$$R = \overline{R} + r, \qquad C_0 = \overline{C_0} + c \tag{9}$$

The regular solution is described by the following equations:

$$\frac{\mathrm{d}}{\mathrm{d}t} \left[ \xi (\overline{C_0} - C_{\mathrm{m}}) \right] = \bar{R} (C_{\mathrm{m}} - K \overline{C_0}) \tag{10}$$

and

$$\tau \frac{\mathrm{d}\bar{R}}{\mathrm{d}t} + \bar{R} = \Re(\overline{C_0}) \tag{11}$$

We assume next that  $\xi$  has the same value for both the full and the regular solution. This is not strictly valid for any kind of perturbation and essentially depends on the characteristic frequency of the oscillation when it exists. The perturbation is associated with a 'penetration depth' which increases as the

oscillation frequency decreases. In other words, the above assumption may be wrong if the oscillatory zoning period is greater than the characteristic time for crystal growth. Clearly, however, the zoning wavelength is much smaller than typical crystal dimensions. Neglecting second-order terms, we get:

$$\xi \frac{\mathrm{d}c}{\mathrm{d}t} + c \frac{\mathrm{d}\xi}{\mathrm{d}t} = \bar{R}(-Kc) + r(C_{\mathrm{m}} - K\overline{C_{0}})$$
 (12)

and

$$\tau \frac{\mathrm{d}r}{\mathrm{d}t} + r = -\frac{\rho}{C_0}c\tag{13}$$

with

$$\rho = -\overline{C_0} \frac{\mathrm{d}\mathcal{R}}{\mathrm{d}C_0} \bigg|_{C_0 = \overline{C_0}} \tag{14}$$

where the sign has been chosen so that  $\rho$  is positive, as  $\mathcal{R}$  is a decreasing function of  $C_0$ .

Equations (12) and (13) are the two perturbation equations to be solved. They are written with  $\bar{R}$  and  $\bar{C}_0$  as parameters and thus describe the perturbations r and c with respect to the quasi-stationary evolution of the system.

The system can be described qualitatively in the (R, C) phase plane (Fig. 4). There are two general types of trajectories in this plane: either a direct convergence or a spiral towards the regular point  $\{\bar{R}, \overline{C_0}\}$ . In terms of growth behaviour, this means either an ordinary convergence to the regular solution (normal growth) or damped oscillations around mean values  $(\bar{R}, \overline{C_0})$  (oscillatory zoning). These two types of behaviour are possible in different areas of the (R, C) plane and thus at different stages of growth.

We now discuss the system in a more quantitative way. The solutions  $\{r, c\}$  of equations (12) and (13) represent either an exponential decay or damped sinusoidal oscillations depending on the sign of the discriminant:

$$\Delta = \left[\frac{1}{\tau} - \frac{1}{\xi} \left( K\bar{R} + \frac{\mathrm{d}\xi}{\mathrm{d}t} \right) \right]^2 - \frac{4\rho}{\xi\tau} \left( \frac{C_{\mathrm{m}}}{\bar{C}_0} - K \right) \tag{15}$$

As K is <1, quantity  $((C_m/\overline{C_0})-K)$  is positive when growth begins  $(\overline{C_0}=C_m)$  and remains positive if  $C_m$  is greater than  $C_S$  (we do not consider the 'hypersaturation' case  $C_m < C_S$ , which is more complex). As growth proceeds,  $\overline{R}$  and  $d\xi/dt$  decrease while  $\xi$  increases from 0 to  $\infty$ . Therefore, the term in brackets in equation (15) increases monotonously from  $-\infty$  to  $1/\tau$ , passing through zero when:

$$\xi = \tau \left( K\bar{R} + \frac{\mathrm{d}\xi}{\mathrm{d}t} \right) \tag{16}$$

The discriminant is thus positive when growth begins, becomes negative, and then positive again. This shows that oscillatory behaviour will happen at some intermediate stage of the crystal history (Fig. 4). The time  $\hat{i}$  when equation (16) is verified marks the transition from the initial growth regime when growth rate is

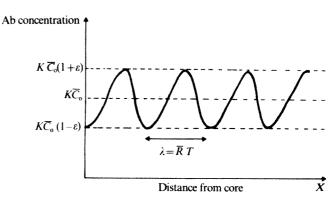


Fig. 5 When the required conditions are met (see text), the perturbation equations result in almost undamped, dissymmetric oscillations in the concentration profile.

essentially determined by the interface kinetics to the final regime when chemical diffusion is the main limiting factor.

However, oscillatory zoning will be detectable only if damping is not too large. The damping coefficient k is defined simply as the ratio of the concentration amplitude from one cycle to the next: k reaches a minimum  $\hat{k}$  at a time close to  $\hat{i}$  defined above. We find that damping is small ( $\hat{k}$  close to unity) when at that time  $\bar{R}/\rho$  is much lower than ( $(C_m/KC_0)-1$ ). As shown by equation (14), this means a high sensitivity of the crystal interface to concentration changes. Consider, for example, equation (5) for  $\Re(C_0)$ . This yields:

$$\frac{\bar{R}}{\rho} = \frac{1}{n} \left( \frac{C_L}{\overline{C_0}} - 1 \right). \tag{17}$$

In this simplified case, oscillatory zoning is certainly detectable for very weak supersaturations as the low damping condition is fulfilled throughout the crystallization sequence.

Furthermore, a long oscillatory episode requires a large value for  $\tau$ . This critical parameter is a measure of the difficulty of rearranging the crystal surface when composition changes. For the plagioclase system, the excess free energy of mixing exhibits large variations in the range  $An_{30} - An_{90}$  because of a complex substitution process due to a transition between a disordered 7 Å and an ordered 14 Å crystal framework (ref. 25, p. 135).

In these conditions, low amplitude, almost undamped, oscillatory zoning is described by:

$$C_{\rm s}(t) = K\bar{C}_0 (1 + \varepsilon \sin \omega t)$$

compared with interface position:

$$X(t) = X(0) + \bar{R}t + \varepsilon \frac{\rho}{\tau \omega^2} \sin \omega t$$
 (18)

where  $\varepsilon$  is the perturbation amplitude. Albite concentration is thus a dissymmetric periodic function of distance X, decreasing more abruptly than it rises (Fig. 5), in agreement with observations<sup>6.7</sup>. The higher the perturbation amplitude  $\varepsilon$ , the more pronounced the dissymmetry.

The above discussion has clarified the conditions for oscillatory behaviour, which seems to be a delicate phenomenon most likely when growth rates are small. We now give numerical estimates for the various parameters.

Equation (1) may be approximated by:

$$D\frac{\partial^2 \bar{C}}{\partial x^2} + \bar{R}\frac{\partial \bar{C}}{\partial x} = 0 \quad \text{in a region } x < x_0 \text{ (close to the interface)}$$

and by

$$D\frac{\partial^2 C}{\partial x^2} = \frac{\partial C}{\partial t}$$
 in a region  $x > x_0$  (far from the interface)

This shows that a reasonable approximation to the concentration profile close to the interface is:

$$\bar{C} = C_{\rm m} + (\bar{C}_0 - C_{\rm m}) \cdot \exp\left(-\frac{\bar{R}}{D} \cdot x\right)$$

Thus  $D/\bar{R}$  is a reasonable estimate for  $\xi$ . We now summarize the quantitative aspects of our theory. We denote by T the oscillation period. The zoning wavelength  $\lambda$  is equal to  $\bar{R}T$ . The damping factor k is taken as  $\hat{k} = \exp{(T/\tau)}$  and small damping implies that ratio  $T/\tau$  is small. This leads to the constraint:

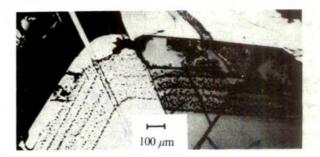
$$\tau \left( K\bar{R} + \frac{\mathrm{d}\xi}{\mathrm{d}t} \right) \simeq \xi \simeq \frac{D}{\bar{R}}$$

Making the further approximation that  $d\xi/dt \ll \bar{R}$ , we obtain the following set of conditions:

$$\lambda = \bar{R}T \simeq 10 \ \mu \text{m}; \quad \bar{R}^2 \tau \simeq \frac{D}{K} \simeq 10^{-8} \ \text{cm}^2 \ \text{s}^{-1} \quad (\text{refs 6, 28})$$

and  $T/\tau$  small (<0.1, say).

The numerical analysis requires a value for the growth rate in realistic conditions. The only measurements available are those



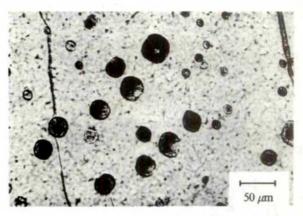


Fig. 6 Rounded glassy inclusions within two different oscillatory zoned plagioclases from North Atlantic tholeittic basalts. Note that, despite the presence of inclusions, oscillatory zones remain remarkably straight and parallel (photographs from ref. 12).

of Lofgren<sup>15</sup> who made plagioclase growth experiments at comparatively low temperatures (800–1,000 °C) and over rather short times (between 10 and 70 h). Taking, for example, a value of 10<sup>-7</sup> cm s<sup>-1</sup>, a value actually measured by Lofgren<sup>15</sup>, we obtain:

$$\tau = 10^6 \,\text{s}$$
 and  $T/\tau = \ln k = 10^{-2}$ 

A value of  $10^{-6} \,\mathrm{cm}\,\mathrm{s}^{-1}$  for the growth rate would yield the following estimates:

$$\tau = 10^5 \, \text{s}$$
 and  $T/\tau = 0.1$ 

The characteristic time  $\tau$  remains poorly constrained but is probably quite large. It may depend on the degree of supersaturation and the estimates quoted above are only valid in the low supersaturation limit.

#### Discussion

Competition between chemical diffusion and interface kinetics should affect all crystals which grow from a melt which is not pure. Delayed response of the growth rate to concentration changes should also be universal. The growth equations predict an episode of oscillatory zoning for all systems provided that the supersaturation is not too large  $(C_s < C_m < C_L)$ . This may seem strange as oscillatory zoning seems to be the exception rather than the rule. Of course, equation (4) is only one way that a time delay could express itself but the explanation is more likely to lie in the numerical values of the characteristic parameters involved in practical situations. For a system in given (Cm, temperature) conditions, oscillatory behaviour is predicted over a certain interval  $\Delta t$  around a time  $\hat{t}$  which marks the transition between interface- and diffusion-controlled growth. For most systems this transition happens much too early or much too late to be detected practically with the result that these systems are classified as interface- or diffusion-controlled. Also  $\Delta t$  must be large enough for oscillations to occur on a measurable crystal length. The oscillation period T must be small enough for the transition episode to include a reasonable number of oscillations and large enough for the zonations, of wavelength  $\lambda = \bar{R}T$ , not to be erased by subsequent solid diffusion. A precise

investigation of these conditions requires a quantitative analysis of the regular solution  $\{\vec{R}(t), \overline{C_0}(t)\}\$  which is beyond the scope of this article. We simply suggest that the plagioclase-melt system is one of the rare systems which fulfill these requirements.

But we have also shown that these requirements were not sufficient: for oscillatory behaviour to be more than an academic prediction, the damping coefficient k must be close to unity, and this in turn requires that the supersaturation be low enough. This explains why oscillatory zoning is common in phenocrysts of volcanic rocks and apparently absent or extremely rare in laboratory experiments<sup>29</sup>. Only when a magma body cools very slowly can plagioclase crystals come to an appreciable size in very low supersaturation conditions. Our quantitative model is certainly oversimplified. More laboratory experiments are needed to improve our understanding of plagioclase growth kinetics, especially in view of the variety and complexity of zoning patterns. That plagioclases are often more complicated than other minerals probably implies that they record a longer history, long residence times being allowed for even in quiescent magmas because of the low plagioclase-melt density contrast<sup>30</sup>.

Remarkably, when oscillatory zoning occurs the planar interface is extremely stable. This is attested by an observation made by Bollinger<sup>12</sup> on oceanic basalts: in these, small glass inclusions

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are sometimes trapped by the growing crystal but do not lead to the formation of dendrites, showing that these lateral perturbations are stabilized (Fig. 6). Such a stability is expected for low supersaturations<sup>31,32</sup>. However, a few samples show contorted bands not conforming to prominent faces11. It would be interesting to investigate how the time delay in equation (4) affects the stability of the interface shape.

#### Conclusions

Our new theory explains the phenomenon of oscillatory zoning which is observed in many naturally grown plagioclase crystals. The theory is based on a simple model of the interface kinetics sensitivity to concentration changes in the melt. The mechanism suggested is probably very general but its effects can only be observed in specific conditions. We believe that an oscillatory zoned plagioclase is a pathological case of crystal growth which enables us to study a fundamental process, that is the response of the crystal growth rate to concentration changes. This process may be responsible for some other characteristics of crystal growth which remain poorly understood, such as the stability of a planar interface.

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## Glucocorticoids regulate expression of dihydrofolate reductase cDNA in mouse mammary tumour virus chimaeric plasmids

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Fusions between the mouse mammary tumour virus long terminal repeat and a mouse dihydrofolate reductase cDNA have been constructed in a SV40 vector. When these plasmids are transferred into recipient cells, the production of dihydrofolate reductase is regulated by glucocorticoid hormones. These results define a hormonally responsive region of the viral genome.

ALL steroid hormones seem to function largely via a common molecular mechanism in which the hormone first associates with a specific soluble receptor protein present in target tissues<sup>1,2</sup>. This interaction causes an alteration in the receptor that increases its affinity for binding sites in the cell nucleus<sup>3,4</sup>. The net effect of steroid action is to alter the pattern of proteins synthesized by the cell, probably by altering the transcription of a few specific genes<sup>5</sup>. It is widely documented that steroidreceptor complexes have DNA-binding properties<sup>2</sup> and that nuclear translocation reflects association of the complexes with

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DNA<sup>6</sup>. Furthermore, both genetic and biochemical evidence has suggested that DNA binding may be involved in eliciting a biological response<sup>7</sup>. To date, however, there is no direct evidence that interactions with DNA lead to changes in transcription or that steroid-receptor complexes must interact with particular DNA sequences to alter gene expression.

We have used mouse mammary tumour virus (MMTV)infected cells as a model to study the regulation of specific gene expression by steroid hormones<sup>8</sup>. When integrated into host cell DNA, the viral genome (the provirus) is transcribed by cellular RNA polymerase II<sup>9</sup> and production of viral RNA is stimulated by glucocorticoid hormones such as dexamethasone 10. Several

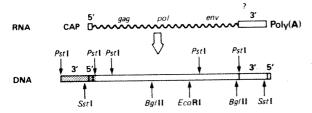


Fig. 1 The structure of the RNA and DNA forms of the MMTV genome. The RNA (~9 kilobase [kb]) form is shown with the putative coding regions indicated: gag, group-specific antigens, core proteins; pol, viral reverse transcriptase; env, viral envelope glycoprotein; ?, a postulated but unidentified gene product<sup>14</sup>. The linear form of MMTV proviral DNA is shown below; on integrating into cell DNA, this structure (with minor modifications at the ends) is maintained. At each end is a LTR consisting of sequences coding for both the 5' and 3' ends of viral RNA; these are shown as the boxes with the structure 3' (~1,200 bp)/5' (130 bp). The PsII fragment from the left-hand end (shown stippled) contains all but a few base pairs of one LTR in addition to 135 bp coding for RNA beyond the 5' leader or 'strong-stop' sequence. This PsII fragment was used as the MMTV fragment in construction of the plasmids shown in Fig. 2.

lines of evidence suggest that hormonal stimulation of MMTV RNA synthesis is mediated by the same intracellular glucocorticoid receptors that are involved in regulation of cellular genes<sup>11</sup>. Like other retroviruses, MMTV DNA integrates at many sites, perhaps even randomly, in the host genome<sup>12</sup>. Studies of viral RNA production in many different clones of MMTV-infected cells suggest that hormonal regulation of MMTV gene expression is not site specific; indeed, there are multiple cellular sites where the provirus is under hormonal control<sup>12</sup>. This argues against the hypothesis that regulation of viral gene expression occurs as a result of integration into a hormonally regulated site on the host chromosome.

In the present studies we sought to determine whether MMTV encodes its own promoter as well as a region required for glucocorticoid responsiveness. Our approach is based on one used successfully in the study of bacterial gene regulation, entailing the construction of a gene fusion between the regulatory region from the gene of interest and the coding region of a second gene whose activity can be conveniently assayed and whose function can be selected for *in vivo*. The expression of the fused gene may then be subject to the control signals present on the regulatory region.

As with other retroviruses, MMTV proviral DNA is bounded by two identical regions composed of sequences derived from both the 5' and 3' ends of the viral genome. These regions are called long terminal repeats (LTRs) and in MMTV are ~1,200 base pairs (bp) long (Fig. 1). The location of the 5' end of MMTV RNA corresponds to a site represented by the junction of the 3' and 5' sequence in the left LTR (Fig. 1), suggesting that the promoter for viral transcription lies within this 1,200-bp region. We have constructed fusions between the MMTV LTR and a cDNA containing the coding region for mouse dihydrofolate reductase (DHFR); the expression of this enzyme can be selected for in the appropriate host cell. These fusions were constructed in a plasmid DNA vector which allows expression of inserted genes after these molecules are introduced into recipient cells<sup>13</sup>. Analyses of the expression and hormonal regulation of these recombinant molecules have allowed us to identify the LTR of MMTV as a region which confers glucocorticoid responsiveness.

## Construction of MMTV-dhfr recombinant plasmids

The PstI fragment from the left end of the viral DNA encompasses an entire LTR (except for 5-10 bp at the extreme left-hand end). The cap site on the 5' end of MMTV RNA is located ~270 bp from the right-hand end of the PstI fragment (ref. 14 and J. Majors and H. Varmus, personal communication); transcription proceeds in the right-hand direction (Fig. 1). It therefore seemed probable that both the promoter for viral RNA synthesis and the sequences involved in hormonal regulation are located within this fragment. We therefore converted the ends of

this 1,450-bp fragment (see Fig. 2 legend) by addition of *HindIII* 'linkers' to facilitate its insertion into the unique *HindIII* site of the vector pSV2dhfr (Fig. 2a). This is a hybrid vector composed of sequences from the papovavirus SV40, the *Escherichia coli* plasmid pBR322 and cDNA coding for the mouse DHFR<sup>15</sup>. Sequences derived from pBR322 allow cloning and propagation of the plasmid in *E. coli* while those derived from SV40 contain processing signals allowing production of a functional messenger RNA for DHFR within mammalian cells<sup>16</sup>.

Individual colonies harbouring the resulting plasmid were screened to identify molecules containing the MMTV fragment oriented in the same direction as the DHFR-coding region. Such a configuration of the MMTV-dhfr fusion would allow transcription initiating at a promoter within the viral fragment also to transcribe dhfr. This plasmid, designated pSVMdhfr, is shown in Fig. 2b. In addition, a derivative of pSVMdhfr, constructed by deleting the SV40 fragment which contains the early promoter (Fig. 2c) and called pMTVdhfr, retains the putative MMTV promoter and regulatory region.

## Transformation characteristics of dhfr-containing plasmids

Dihydrofolate reductase catalyses the formation of tetrahydrofolate, a cofactor essential for many one-carbon transfer reactions such as in the biosynthesis of glycine and thymidine. A cell line<sup>17</sup> derived from Chinese hamster ovary (CHO) cells provides an ideal recipient for the *dhfr* vectors in DNA transformation experiments. These cells are defective in DHFR and, unlike wild-type cells, are unable to grow in medium lacking glycine,

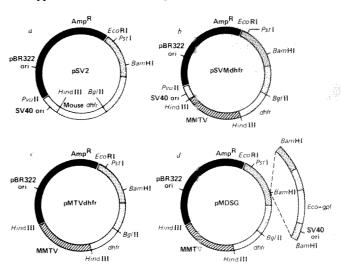


Fig. 2 Structure of plasmid vectors containing mouse dhfr and E. coli gpt. The solid black segment of each plasmid is a 2.3-kb fragment of pBR322 extending from the EcoRI site to the PvuII size which contains the lactamase gene (ampicillin resistance) and the origin of replication. The lightly stippled region of pSV2, pSVM and pMDSG between the PvuII and HindIII sites is a 325-bp fragment of SV40 (coordinates 0.65-0.71) containing the early promoter and the SV40 origin of replication. The other stippled regions are SV40 sequences encoding the intron for small T antigen (lightly stippled, SV40 coordinates 0.44–0.56) and the site for polyadenylation for early mRNA (heavily stippled, SV40 coordinates 0–0.19). All are described in ref. 13. The hatched region on pMTV, pSVM and pMDSG is the MMTV LTR. The open regions are either mouse dhfr cDNA15 or E. coli gpt13. Transcription from both the SV40 early promoter and the MMTV promoter is in the anticlockwise orientation, as are the coding sequences of dhfr and gpt. The sizes of the fragments are drawn only approximately to scale. Derivatives of pSV2dhfr were constructed as follows: The 1.4-kb PsrI MMTV LTR fragment was treated with T4 DNA polymerase to remove protruding ends, then HindIII linker fragments were added by ligation with T4 ligase. This fragment was inserted at the unique HindIII site of pSV2dhfr separating the SV40 origin fragment from dhfr sequences to produce pSVMdhfr pMTVdhfr (c) was generated by digesting pSV2dhfr with Pvull and HindIII. adding HindIII linkers to the Poull site and inserting the modified MMTV fragment. Colonies were screened to isolate molecules containing the MMTV DNA in the appropriate orientation pMDSG (d) was constructed by inserting a 2.2-kb BamHI fragment containing the SV40 origin/promoter fragment fused to Eco-gpt and SV40 RNA processing signals into the unique BamHI site of pMTVdhfr. This 2.2-kb fragment was derived by addition of a BamHI linker to the PvuII sitesof pSV2gpt13

Table 1 Transformation efficiencies and hormonal regulation of dhfr-containing plasmids in dhfr CHO cells

Vector	Structure	Transformation frequency	Hormonal regulation		
pSV2dhfr	SV40-dhfr	$1-2 \times 10^{-4}$	-	(0/4)	
pSVMdhfr	SV40-MTV-dhfr	$1 \times 10^{-4}$	+	(5/5)	
pMTVdhfr	MTV-dhfr	$1 \times 10^{-6}$	+	(3/3)	
pMDSG	MTV-dhfr: SV40-gpt	$1 \times 10^{-4}$	+	(3/3)	
pSVMdhfr'	SV40-VTM-dhfr	$1-2\times10^{-5}$	***	(0/4)	
pSV0dhfr	dhfr	$\leq 1 \times 10^{-7}$	Not de	termined	

Transformations were performed using the calcium phosphate co-precipitation method of Graham and van der Eb $^{18}$ . In brief,  $10-15~\mu g$  of circular plasmid DNA were precipitated with CaPO $_4$  and added to  $10^6$  cells growing in non-selective medium (Ham's F12 medium). Four hours after addition of DNA, cells were relycerol shocked' with 20% glycerol in phosphate-buffered saline (PBS) according to the method of Frost and Williams $^{25}$ . Medium was replenished the following day and the cells allowed to grow for 2 more days in non-selective conditions. Cells were then passaged into selective medium (Dulbecco's minimal essential medium  $+34.5~\mu g$  ml $^{-1}$  proline);  $10^6$  cells were plated per 10-cm dish. Medium was changed every 2-3 days and dhfr $^*$  clones became visible 7–10 days later. Transformation frequencies are reported as transformants per cell (for example, 100 colonies on a dish plated with  $10^6$  cells yields a frequency of  $10^{-4}$  transformants per cell). Hormonal regulation was determined either by methotrexate resistance (Fig. 3) or  $^3 H$ -methotrexate binding (Table 2); the numbers in parentheses indicate the number of individual transformants analysed and the proportion which were hormonally responsive. In pSVMdhfr', VTM indicates that the MMTV fragment was inserted in the anti-sense orientation.

thymidine and hypoxanthine. One can identify clones expressing the *dhfr* gene present on the transforming DNA by selecting for clones able to grow in the absence of one or more of the required supplements.

DNA was introduced into the CHO dhfr cells using the transformation procedure of Graham and van der Eb<sup>18</sup>. A calcium phosphate precipitate containing 15  $\mu$ g of plasmid DNA was added to 10<sup>6</sup> cells growing in non-selective medium; after transfer into selective medium, colonies were seen within 7–10 days. When pSV2dhfr DNA was used we observed 100–200 colonies (frequency  $\sim 1-2\times 10^{-4}$  per cell). Similar results have been obtained by Subramani et al. 6. The expression of dhfr in these cells is apparently directed by the SV40 early promoter because very few transformants (frequency  $\leq 1\times 10^{-7}$  per cell) are observed if transformations are attempted with a plasmid (pSV0dhfr) lacking the SV40 promoter/origin fragment (Table 1). PSV0dhfr was generated by deleting the region between the *PvuII* and *HindIII* sites of pSV2dhfr (Fig. 2a).

Similar experiments were performed with pSVMdhfr and pMTVdhfr. As shown in Table 1, the frequency of transformation with pSVMdhfr is similar to that with pSV2dhfr. The possibility exists, however, that the SV40 early promoter, located directly upstream of the MMTV LTR, is responsible for dhfr expression by readthrough transcription. This could occur with or without removal of MMTV sequences via a posttranscriptional splicing event. Results obtained with the pMTVdhfr vector eliminate this possibility because the SV40 promoter is absent. When CHO dhfr cells were infected with this DNA, transformants were indeed obtained, although at a considerably reduced frequency ( $\sim 10^{-6}$  per cell; Table 1). Nevertheless, the ability to isolate dhfr+ colonies following transformation with pMTVdhfr suggests that sequences present on the MMTV LTR are serving a promoter-like function; this is supported by studies of the 5' end of the dhfr RNAs in the transformants (see below).

We considered whether pMTVdhfr is less efficient at producing dhfr<sup>+</sup> colonies because the MMTV promoter is intrinsically inefficient. Such a weak promoter might produce levels of dhfr RNA only marginally sufficient to support growth in selective medium, thereby leading to reduced numbers of transformants. Thus we constructed another vector derived from pMTVdhfr in which the MMTV-dhfr fusion could be introduced into cells without directly selecting for dhfr expression; DHFR production might be detectable in the CHOdhfr<sup>-</sup> cells even if those levels were not sufficient to support growth. This vector, designated pMDSG (Fig. 2d), contains a DNA fragment that encodes the E. coli xanthine-guanine phosphoribosyl transferase (gpt)

gene under SV40 early promoter control inserted at the BamHI site of pMTVdhfr. The expression of the E. coli gene can be selected for in the CHOdhfr cells using the dominant selection procedure described by Mulligan and Berg<sup>13</sup>. The frequency of gpt\* clones obtained with pMDSG approximates the frequency of dhfr<sup>+</sup> transformants obtained with pSV2dhfr or pSVMdhfr  $(1 \times 10^{-4} \text{ per cell})$ . Three individual clones were picked, grown to mass culture and transferred to DHFR-selective medium. Surprisingly, all three clones grew as well as other dhfr transformants (data not shown). Furthermore, when pMDSG was used to transform cells selecting for dhfr directly, transformants also arose at a frequency of  $\sim 1 \times 10^{-4}$  per cell (Table 1). The relatively high level of transformation with pSVMdhfr and pMDSG, compared with that with pMTVdhfr, seems to be due to the presence of the SV40 fragment encompassing the promoter and origin of replication (S. Subramani and P. B. M. Fromm and P.B., F.L. and G.R., unpublished observations). This novel effect of the SV40 promoter/origin region on the efficiency of DNA mediated transformation will be described in greater detail elsewhere.

#### Expression and glucocorticoid regulation of dhfr in transformants

Regardless of differences in transformation frequencies, our results suggest that the dhfr cDNA can be expressed when fused to the MMTV LTR. This would be consistent with the presence of a promoter on this fragment of MMTV DNA, perhaps near the cap site. Our major interest in these experiments was to ascertain whether the expression of dhfr in these MTV-dhfr fusions could be regulated by glucocorticoids. A positive answer would suggest that the viral DNA indeed carries its own regulatory site.

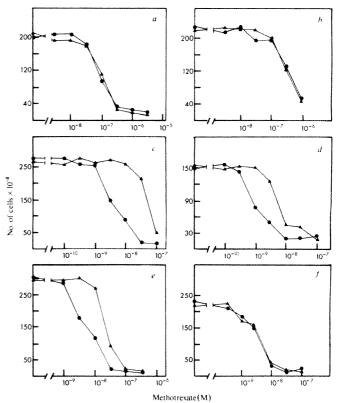


Fig. 3 Methotrexate inhibition of CHOdhfr cells transformed with dhfr cDNA-containing plasmids. Individual clones of CHOdhfr cells arising from transformation with the indicated plasmids were grown in selective medium (Dulbecco's minimal essential medium + 39.5 µg ml<sup>-1</sup> proline). In each case, ~10<sup>5</sup> cells were plated on 6-cm dishes either in the presence or absence of 10<sup>-6</sup> M dexamethasone. Twenty-four hours later, methotrexate was added to individual dishes at the indicated concentrations. Fresh medium containing methotrexate was added after 2 days and the surviving cells counted after 2 more days. a, Wild-type CHOKI cells; b, transformant pSV2dhfr.1; c, transformant pMTVdhfr.2; d, transformant pMDSG.8; e, transformant pSVMdhfr.4; f, transformant pSVMdhfr'.6. Dexamethasone absent (•) or present (•).

Table 2	<sup>3</sup> H-methotrexate binding of cell extracts					
	c.p.m. <sup>3</sup> H-metho					
Transformant	-Dex	+Dex	Fold induction			
pSV2dhfr.1	9.882	8,745	0			
pMTVdhfr.1	116	571	5			
pMTVdhfr.2	444	1.148	3			
pMTVdhfr.3	972	5,111	5			

Cells of individual clones were grown in Dulbecco's minimal essential medium + proline in the presence or absence of  $10^{-6}$  M dexamethasone (Dex) for 2 days. Cells were removed from dishes with PBS/EDTA and washed once with PBS.  $1-2\times10^7$  cells were resuspended in 1 ml of 0.05 M potassium phosphate buffer pH 7.4. The suspended cells were frozen and thawed three times in a dry ice/ethanol bath, and then centrifuged for 15 min in a microfuge.  $^3$ H-methotrexate (Amersham, 200 mCi mmol $^{-1}$ ) was added to equal amounts of extract from each cell line ( $\sim300~\mu$ g total protein in 50  $\mu$ l) and allowed to bind in the dark for 10 min at 25 °C. The mixture was passed over an 8-ml Sephadex G-50 column equilibrated in 0.01 M KPQ4 pH 6, 0.15 M KCl. The counts in the excluded volume were determined by counting in a scintillation spectrometer. In subsequent experiments we observed four- to fivefold inductions in all three pMTVdhfr clones.

Initially we attempted to measure DHFR in pMTVdhfr transformants using a standard enzymatic assay which measures conversion of <sup>3</sup>H-folate to <sup>3</sup>H-dihydrofolate and tetrahydrofolate. However, the levels of the enzyme are too low to be detected reproducibly in these cells using such an assay (F.L. and G.R., unpublished results). We therefore used methotrexate, a folate analogue which inhibits DHFR and thus inhibits cell growth, to estimate levels of the enzyme. Cells were plated in various concentrations of methotrexate, in either the presence or absence of dexamethasone, and the number of cells on each dish determined 4-5 days later. Figure 3a shows the results of such an experiment using wild-type CHO-Kl cells. The concentration of methotrexate required to inhibit growth of these cells by 50% is  $\sim 10^{-7}$  M; dexamethasone has no effect on the cells' sensitivity to methotrexate. Similar results are observed when pSV2dhfr transformants of the CHOdhfr cells are examined (Fig. 3b). Again, methotrexate sensitivity is unaffected by hormone treatment; this is true of four independent pSV2dhfr transformants we have examined.

In contrast, in experiments using pMTVdhfr transformants, the cells were considerably more resistant to methotrexate in the presence of dexamethasone than in its absence (Fig. 3c); the hormone-treated cells are resistant to  $\sim 10-20$ -fold more methotrexate. Furthermore, the levels of methotrexate required to inhibit growth of these cells  $(10^{-9}-10^{-8} \, \mathrm{M})$  is lower than that required to inhibit pSV2dhfr transformants. We believe this indicates that the MMTV promoter is less efficient than either the SV40 early or the cellular dhfr promoter.

In cells transformed with pMDSG DNA, dexamethasone also increases the resistance to methotrexate (Fig. 3d). Similar results have been obtained with several clones of pSVMdhfr transformants (Fig. 3e). Indeed, all cells transformed with a plasmid containing dhfr cDNA fused to the MMTV LTR in the proper orientation exhibit an approximate 10-fold increase in methotrexate resistance when grown in the presence of dexamethasone. If, however, we use a vector in which the MMTV LTR is inserted into pSV2dhfr in the opposite orientation relative to the dhfr cDNA (pSVMdhfr'), resultant transformants do not respond to dexamethasone (Fig. 3f). We do not know whether the dhfr expressed in these cells arises from a promoter on the opposite strand of the MMTV LTR. Nevertheless, the results of all the experiments indicate that the MMTV LTR is required for hormonal regulation of dhfr and that it must be in the proper orientation (Table 1).

Previous studies<sup>19</sup> have indicated the lack of a 1:1 correlation between the amount of DHFR present in a cell and a cell's sensitivity to methotrexate. To obtain a more quantitative estimate of the levels of DHFR in the transformed cells, we have measured <sup>3</sup>H-methotrexate binding to DHFR in cell extracts. Table 2 summarizes the results of such an experiment. pMTVdhfr-transformed cells clearly contain three- to fivefold more DHFR when grown in the presence of dexamethasone than in its absence; as described above, this results in a 10-20-fold increase in methotrexate resistance. Cells transformed with

pSV2dhfr contain higher levels of DHFR; however, these levels are unaffected by the hormonal status of the cell. Similar experiments with extracts from pMDSG- and pSVMdhfr transformants indicate that glucocorticoids result in a consistent three- to fivefold increase in levels of DHFR. All the data are in agreement with the methotrexate growth-inhibition studies.

Note that the level of induction of DHFR in the CHO cells is somewhat lower than the average 10-fold induction of MMTV RNA in mouse mammary tumour cells and much lower than the 50-500-fold level of induction of MMTV RNA in infected HTC, rat hepatoma cells. The reasons for these differences are obscure, although we have observed that the concentration of glucocorticoid receptors in the CHO cells is  $\sim 5-10\%$  that of HTC cells. We are investigating whether the induction of dhfr or other gene fusions can be augmented in cells with higher levels of receptor. Indeed, recent experiments in mouse 3T6 cells using pSVM plasmids containing the Eco-gpt gene rather than dhfr cDNA, indicate that production of xanthine-guanine phos-

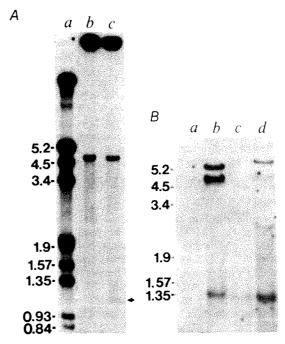


Fig. 4 A, S<sub>1</sub> nuclease mapping of MTV-dhfr RNA made in transformant pMTVdhfr.2. The <sup>32</sup>P-end-labelled probe for the hybridization was prepared as follows. pMTVdhfr plasmid was cut at the unique Bg/II site at the end of the dhfr cDNA (see Fig. 2). After treatment with alkaline phosphatase and labelling the 5' ends with <sup>32</sup>P-ATP and T4 polynucleotide kinase, the linear DNA was digested with EcoRI. The two resulting fragments were run on an agarose gel and the 4.5-kb EcoRI-BglII fragment containing the MMTV LTR and dhfr cDNA labelled at the Bg/II end was isolated from agarose. Total cytoplasmic RNA was isolated from pMTVdhfr.2 cells grown with or without dexamethasone  $(10^{-6})$  as described previously26. Poly(A)-containing RNA was isolated by binding to oligo(dT) cellulose. Approximately 5  $\mu g$  of poly(A)-containing RNA was hybridized with an excess of end-labelled DNA fragment in 80% formamide according to the method of Berk and Sharp<sup>20</sup>. Hybridization was performed at 50°C for ~16 h. Hybrids were treated with S<sub>1</sub> nuclease (Boehringer-Mannheim) and run on a 1.4% agarose gel which was autoradiographed for 7 days. a,  $^{32}$ P-end-labelled  $\lambda$  DNA (digested with EroRI and HindIII; sizes are indicated in kb); b, MTVdhfr.2 RNA minus dexamethasone; c, MTVdhfr.2 RNA plus dexamethasone. The arrow indicates the ~1-kb RNA-DNA hybrid. The band in b and c at -4.5 kb is the reannealed EcoRI-Bg/II probe. The dark band at the top of b and c represents material that did not enter the gel. B, Analysis of pMTVdhfr DNA in three dhfr\* clones. Three independent dhfr<sup>+</sup> clones arising following transformation with pMTVdhfr DNA were picked and grown to mass cultures. Total cell DNA was prepared from each clone as well as from the parental CHOdhfr cells. 10 µg of each DNA was digested to completion with HindIII and run on a 0.8% agarose gel, and then transferred to nitrocellulose by the method of Southern<sup>22</sup> The filter was hybridized with  $-5 \times 10^6$  c.p.m. of  $^{32}$ P-nicktranslated pMTVdhfr DNA, washed and autoradiographed as described previously<sup>12</sup>. Order of samples: a, CHOdhfr; b, MTVdhfr.1; c, MTVdhfr.2; d, MTVdhfr.3. The molecular weight markers were unlabelled fragments of DNA cut with EcoRI and HindIII, visualized by ethidium bromide staining. The common band appearing in all the MTV dhfrclones, ~1.4 kb long, is the MMTV LTR fragment which is excised by HindIII (see Fig. 2)

phoribosyl transferase is augmented at least 10-20-fold by dexamethasone (F.L. and G.R., unpublished results).

## Moving the 5' end(s) of MMTV-dhfr RNA

To determine whether the RNA expressed in the pMTVdhfr transformants initiates within MMTV DNA, we analysed the 5' end of the transcripts by the procedure of Berk and Sharp<sup>20</sup>. The Bg/II-EcoRI fragment encompassing the MMTV-dhfr sequences of pMTVdhfr was labelled with <sup>32</sup>P at the Bg/II site. Hybrids between this fragment and poly(A)+ RNA from the cytoplasm of pMTVdhfr.2 cells were treated with S<sub>1</sub> nuclease and analysed by electrophoresis in 1.4% agarose gels. The hybrid band detected by autoradiography is ~1,000 bp long (Fig. 4a), indicating that the predominant 5' end of dhfr RNA maps to a site within the MMTV LTR, 250-275 bases upstream of the dhfr insert. Furthermore, the production of this RNA is stimulated in cells treated with dexamethasone.

The S<sub>1</sub> nuclease analysis shown in Fig. 4a contains a heterogeneous background of apparent hybrids 1.0-4.5 kbp long. These seem to be artefacts of incomplete S<sub>1</sub> digestion rather than random sites of initiation; the long exposure time required to see the hybrid band has made it technically difficult to overcome this problem. However, transformants grown in progressively increasing concentrations of methotrexate amplify the MMTV-dhfr fusion and overproduce the dhfr RNA several hundredfold21. S1 nuclease analysis of RNA from such cells indicates that >90% of the dhfr RNA molecules initiate at the same site within the MMTV LTR as that shown by the 1kilobase band in Fig.  $4a^{21}$ . In these conditions the background is eliminated because much shorter exposure times (4 h compared with 7 days) are required to visualize the authentic hybrid.

### Integration of pMTVdhfr DNA in transformants

To determine the fate of the plasmid DNA used for transformation of the CHOdhfr cells, we have analysed cellular DNAs from pMTVdhfr transformants by the procedure of Southern<sup>22</sup>. Cell DNAs were cleaved with HindIII, which cleaves pMTVdhfr twice, and the fragments resolved by agarose gel electrophoresis. After transfer of the DNA onto nitrocellulose filters, the plasmid sequences were detected by hybridization with pMTVdhfr DNA labelled with 32P by nick-

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translation. As seen in Fig. 4b, the three independently isolated transformants harbour plasmid-related sequences in different locations within cell DNA, as judged by the different mobilities of the hybridizing bands. This is corroborated by analysis of DNAs digested with EcoRI (data not shown).

We have not yet mapped the sites of integration either within the host or the plasmid DNAs. Interestingly, in this limited sample of clones there seem to be only one to two copies of integrated vector DNA in the transformants. We have no evidence of free plasmid DNA being maintained within the pMTVdhfr-transformed CHO cells.

#### **Conclusions**

The results of the experiments described here provide evidence that the MMTV LTR contains sequences which are sufficient for glucocorticoid regulation of gene expression. This extends the observations of others that the entire MMTV genome is glucocorticoid sensitive when introduced into mouse L cells by DNAmediated transformation<sup>23,24</sup>. Whether glucocorticoids are acting by regulating transcription per se remains to be established. The LTR fragment we have used contains ~270 bp which encode the 5' end of MMTV RNA, and differences in RNA processing within this region may account for hormonal regulation of MMTV RNA production in virus-infected cells or of dhfr in the transformants described here. In addition, there may be other viral sequences outside the LTR or cellular sequences which contribute to the hormonal response. Whatever the mechanism(s), we are now in a position to determine which sequences within this MMTV fragment are required for hormonal responsiveness. It will be of interest to determine whether these sequences serve as specific recognition sites for the glucocorticoid-receptor complex.

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## Hexose transport in hybrids between malignant and normal cells

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The kinetic parameters of hexose uptake were measured in matched pairs of hybrids between malignant and normal cells. Each pair consisted of a hybrid in which malignancy was initially suppressed and a segregant tumour derived from it. Comparisons were also made between tumour cells and non-tumorigenic derivatives selected from the tumour cell populations in vitro. Without exception, malignancy, as defined by the ability of the cell to grow progressively in vivo, was found to be linked to a systematic decrease in the Michaelis constant of the hexose transport system.

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FOLLOWING the initial observation of Hatanaka et al.<sup>1</sup>, many authors have reported that morphological transformation of fibroblastic cells in vitro by certain oncogenic viruses is associated with an increase in the uptake of glucose by the cell. Although conflicting reports exist, the majority view is that the increase in uptake is due to an increase in the  $V_{\rm max}$  for glucose transport. Occasional reports of a reduction in the  $K_{\rm m}$  of the glucose uptake<sup>2,3</sup> have been difficult to reproduce<sup>4,5</sup>. There has been little work, however, and no formal kinetic study, on the relationship between glucose uptake and tumorigenicity.

Recent observations made in this laboratory have rekindled our interest in this problem. Bramwell and Harris<sup>6</sup> have shown that malignancy in hybrid cells is systematically linked to a structural alteration in the carbohydrate moiety of one particular membrane glycoprotein. Subsequent experiments provided strong circumstantial evidence that this protein had some role in glucose transport<sup>7,8</sup>. Gingrich et al.<sup>9</sup> have shown that a monoclonal antibody raised against partially purified preparations of the protein described by Bramwell and Harris delineates an antigen that behaves in a range of physiological tests as if it were the glucose transport protein. It was therefore of interest to see whether the structural change described by Bramwell and Harris was associated with any systematic functional alteration in hexose transport. Our approach has again been to compare hybrid cells in which malignancy is initially suppressed with malignant segregants derived from these hybrids. Isogeneic matched pairs of this kind provide a sensitive screen for markers linked to malignancy 10-14. We have also examined some non-malignant variants derived from tumour cells by selection for resistance to the lectin wheat-germ agglutinin°.

#### Hexose transport measurement

Hexose transport was measured by the uptake of 2-deoxy[ $^3$ H]glucose (1–70 mCi mmol $^{-1}$ , Amersham) in the following conditions. The cells were inoculated into 16-mm diameter tissue culture wells 24 h before assay. Each well received 2 ml of Eagle's minimum essential medium supplemented with 5% fetal calf serum and 5% newborn calf serum in which the cells were suspended at a concentration of 5– $15 \times 10^4$  per ml. The adherent monolayers were washed twice in phosphate-buffered saline PBS and 500  $\mu$ l of the radioactive sugar in PBS were added at 20 °C. Samples were collected at appropriate times by dissolving the cell monolayer, after two washes in PBS, in 440  $\mu$ l of 0.4 M NaOH. The extract was neutralized with 10  $\mu$ l of glacial acetic acid, mixed with 10 ml of Unisolve 1 and counted in a Packard scintillation counter.

The rate of uptake was derived from a six-point time course and was measured at four to six concentrations of 2-deoxyglucose in the range 0.1-5 mM. The uptake was linear for at least 10 min. Nonspecific uptake of the radiolabel was estimated by measuring the retention of L-[3H]glucose in parallel conditions, added at a concentration of 0.1 mM at the same specific activity per ml as the 2-deoxy[3H]glucose. The mean of the measurements obtained on each of six wells was subtracted from the 2-deoxy[3H]glucose uptake measurements, and the mean number of cells per well estimated by counting the trypsinized contents of six wells in a Coulter counter. For cells in suspension, uptake of the labelled 2-deoxyglucose was measured in 1.5-ml microfuge tubes. The cells were first pelleted by centrifugation for 4 s in a Beckman microfuge, washed once with PBS and then resuspended with the radioactive sugar. Cells were collected by centrifugation followed rapidly by a wash with ice-cold PBS. Allowance was again made for nonspecific retention of the radiolabel.

At each substrate concentration,  $S_i$ , an estimate of the reaction velocity,  $V_i$ , and the variance of that estimate were calculated by linear regression through the origin of the linear portion of the plot of 2-deoxyglucose uptake against time. The  $K_m$  and  $V_{max}$  values and their standard errors were calculated by a weighted linear regression of S/V against V, essentially as described by Cornish-Bowden<sup>15</sup>. The final weight applied to

each  $S_i/V_i$  was  $[V_i^2/V_{ar}V_i][V_{max}^2/(K_m+S_i)^2]$ . Inspection of the residuals in V confirmed that these weightings were acceptable  $^{16}$ .

For some cell lines uptake of D-glucose was measured by the silicone oil filtration centrifugation technique of Werdan et al.<sup>17</sup>. The kinetic constants thus obtained agreed well with those obtained by the uptake of 2-deoxyglucose as described above.

## Kinetic parameters of hexose uptake in parental malignant and normal cells

Table 1 gives the  $K_m$  and  $V_{max}$  values for the parental tumour cells and the diploid cells with which they were fused. The  $V_{
m max}$ values are highly variable, as expected, for it is known that in some cells the  $V_{\text{max}}$  of the hexose uptake is very sensitive to cell density and other conditions of culture. The  $V_{\rm max}$  values for some of the tumour cells are substantially lower than those for the diploid fibroblasts. There is clearly no correlation between tumorigenicity and a high  $V_{\rm max}$  in vitro. On the other hand, the tumour cells and the diploid cells differ consistently in the  $K_{\rm m}$ , the  $K_m$  values for the tumour cells being about half those for the diploid cells. The variation in  $K_m$  in the different fibroblast populations probably represents genotypic variation, as the duplicate values were derived from measurements on cells freshly isolated from two different animals. The PG19 tumour is derived from a C57BL mouse and is syngeneic with the C57BL fibroblasts

The question of whether the reduced  $K_m$  seen in the tumour cells is fortuitous or whether it is closely linked to the genesis of the malignant state can now be explored by comparing the matched pairs of hybrids, as we have described.

### Lymphoma × fibroblast crosses

The origin and characteristics of clone 1G, a hybrid clone derived from the lymphoma×fibroblast cross (YACIR×CBAT6T6 fibroblast), have been described previously<sup>18</sup>. The clone initially produced no tumours even with inocula of  $4\times10^6$  cells per mouse (sublethally irradiated, syngeneic, newborn animals), but after several weeks' cultivation in vitro, a malignant subpopulation was generated that overgrew the cultures. Early passages of clone 1G were recloned and the secondary

Table 1 Kinetic constants of hexose uptakesin malignant and normal cells

Cell type	Cell density (cells cm <sup>-2</sup> )	Passage no.	К <sub>т</sub> (mM)	$V_{ m max}$ (nmol per $10^6$ cells per h)
PG19	$2.5 \times 10^{5}$		$0.608 \pm 0.114$	$96.1 \pm 7.8$
	$1.2 \times 10^{5}$		$0.992 \pm 0.119$	$108.2 \pm 7.8$
YACIR	Suspension culture		$1.101 \pm 0.033$	$44.7 \pm 0.8$
A9HT	$2.1 \times 10^{5}$		$4.170 \pm 0.079$	$131.4 \pm 6.3$
SEWA	Suspension culture		$0.683 \pm 0.008$	$494.7 \pm 2.6$
TA3HaB	$2.0 \times 10^{5}$		$0.762 \pm 0.078$	$291.2 \pm 16.7$
C57BL				
fibroblasts	$4.6 \times 10^{4}$	2	$2.510 \pm 0.232$	$184.0 \pm 12.6$
	$4.8 \times 10^{4}$	5	$2.500 \pm 0.494$	$295.7 \pm 44.7$
CBAT6T6				
fibroblasts	$3.5 \times 10^{4}$	3	$1.622 \pm 0.182$	$255.2 \pm 16.1$
	$9.9 \times 10^{4}$	4	$1.773 \pm 0.153$	$287.9 \pm 13.2$
T13HT13H				
fibroblasts	$1.1 \times 10^{5}$	4	$1.610 \pm 0.388$	$162.8 \pm 19.3$
	$7.1 \times 10^{4}$	4	$1.730 \pm 0.127$	$387.5 \pm 15.9$
Rb7BnR/Rb7BnR fibroblasts	9.4×10 <sup>4</sup>	2	2.153 ± 0.155	184.6±8.6
C57BL				
lymphocytes	Suspension culture		1.740 ± 0.085	$4.5 \pm 0.17$

PG19 is an HGPRT<sup>-</sup> derivative of a melanoma that arose spontaneously in a C57BL mouse. YACIR is a Moloney virus-induced lymphoma; an HGPRT<sup>-</sup> derivative was used. A9HT is a malignant derivative of the A9 cell line selected by passage through the animal. SEWA is a polyoma virus-induced osteosarcoma. TA3HaB is an HGPRT<sup>-</sup> derivative of the TA3 Hauschka cell line (details of these tumours are given in refs 10, 19). The fibroblasts were secondary cultures derived from trypsinized mouse embryos and were assayed at early passages as shown. The lymphocytes were teased from adult mouse spleen and purified by centrifugation in Ficoll-Paque.

Table 2 Kinetic constants of hexose uptake in malignant and non-malignant lymphoma × fibroblast hybrids

Hybrid	Tumori- genicity	Cell density (cells cm <sup>-2</sup> )	К <sub>т</sub> (mM)	$V_{ m max}$ (nmol per $10^6$ cells per h)
Cl1G8	1600	$5.7 \times 10^4$	$3.214 \pm 0.255$	$140.7 \pm 8.9$
Cl1G1	+	Nonline	ar reciprocal plot	
Cl1G1T1	+	$4.7 \times 10^{4}$	$0.725 \pm 0.098$	$106.1 \pm 7.6$
Cl1G1T2	+	$5.5 \times 10^{4}$	$1.088 \pm 0.336$	$86.9 \pm 13.9$
CIIGIT3	+	$8.9 \times 10^{4}$	$1.063 \pm 0.157$	$242.6 \pm 21.9$
Cl1G1A		Nonline	ar reciprocal plot	
CIIGIE		$1.6 \times 10^{5}$	$1.461 \pm 0.208$	$37.7 \pm 3.2$
Cl1G1F		$8.9 \times 10^{4}$	$1.198 \pm 0.165$	$63.1 \pm 4.8$
Cl1G1H		$8.8 \times 10^{4}$	$0.666 \pm 0.107$	$41.6 \pm 3.6$
CliGiJ		$9.3 \times 10^{4}$	$0.938 \pm 0.198$	$57.6 \pm 6.5$
Cl1G8a	+	$7.5 \times 10^4$	$0.851 \pm 0.068$	$74.8 \pm 2.9$
Cl1G8b	+	$1.5 \times 10^{5}$	$1.413 \pm 0.263$	$62.8 \pm 8.5$
Cl1G8c	+	$6.0 \times 10^{4}$	$0.835 \pm 0.047$	$146.1 \pm 4.2$
Cl1G8bT1	+	$5.2 \times 10^4$	$1.281 \pm 0.168$	$135.9 \pm 6.8$
Cl1G8bT2	+	$6.2 \times 10^4$	$1.349 \pm 0.110$	$179.5\pm8.8$

clones tested for tumorigenicity. Two of these secondary clones, 1G1 and 1G8, were selected for further study. 1G8 produced no tumours with inocula of  $5\times10^5$  cells per mouse while 1G1 produced tumours in about 90% of the animals with this inoculum.

Three tumours derived from 1G1 were explanted and the cell populations assayed in vitro (clone 1G1T1, T2, T3). The kinetic constants for clone 1G8 are shown in Table 2. Clone 1G1 gave markedly nonlinear reciprocal plots, but the plots for the tumours derived from 1G1 were linear and their kinetic constants are also shown in Table 2. It is clear that the  $K_m$  for hexose transport of the three 1G1 tumours is substantially lower than that of the non-malignant clone 1G8 (a factor of almost 3).

It seemed reasonable to assume that the curvilinearity of the reciprocal plots for clone 1G1 was due to a mixture of cells in the population assayed. Clone 1G1 was therefore recloned to give tertiary clones 1G1A, E, F, H and J. Clone 1G1A still gave a curvilinear plot, but clones E, F, H and J gave linear plots with the  $K_{\rm m}$  and  $V_{\rm max}$  values shown in Table 2. Each of these clones gave the low  $K_{\rm m}$  characteristic of tumorigenic cells. These results thus support the view that tumorigenic variants are generated in the initially non-malignant cell populations and progressively overgrow the cultures. As no tumours were produced by direct injection of clone 1G8, we attempted to select from this clone subclones capable of growth in agarose, in the hope that such subclones might be enriched for cells capable of progressive growth in vivo. From three dishes, each seeded with 10<sup>5</sup> cells in agarose as described by Steinberg and Pollack<sup>19</sup>, five primary colonies were obtained, of which three survived isolation and further subculture (clones 1G8a, b, c). These gave 80-100% take incidences at  $5 \times 10^5$  cells per mouse. Table 2 shows the  $K_{\rm m}$ and  $V_{
m max}$  values for these clones and for tumours derived from them (1G8bT1 and T2). Both the clones themselves and the tumours produced by them have  $K_m$  values two to three times

Table 3 Kinetic constants of hexose uptake in melanoma × fibroblast hybrids and in a lectin-resistant melanoma derivative

Cell type	Tumori- genicity	Cell density (cells cm <sup>-2</sup> )	К <sub>т</sub> (mM)	$V_{ m max}$ (nmol per $10^6$ cells per h)
PG19×				
T13HT13H		_		
Clone 7		$2.1 \times 10^{5}$	$2.348 \pm 0.339$	$195.0 \pm 16.4$
PG19×				
T13HT13H				
Clone 8		$5.3 \times 10^4$	$3.587 \pm 0.572$	$160.5 \pm 18.4$
		$7.9 \times 10^{4}$	$2.533 \pm 0.466$	$132.5 \pm 16.2$
PG19×				
T13HT13H				
Clone 8T1	+	$2.1 \times 10^{5}$	$1.480 \pm 0.051$	$204.3 \pm 3.8$
PG19WGAR				
Clone C2		2.0×10 <sup>5</sup>	$2.40 \pm 0.230$	173.5 ± 11.5

lower than that of the non-tumorigenic clone 1G8 from which they were derived.

The association between reduced  $K_{\rm m}$  for hexose transport and tumorigenicity is thus not fortuitous. Malignant derivatives obtained directly by the inoculation of the hybrid cell population into the animal or indirectly by selection in semi-solid medium systematically show a reduction in  $K_{\rm m}$  compared with the non-tumorigenic hybrids from which they were derived.

#### Melanoma × fibroblast crosses

Crosses between the PG19 melanoma derivative and diploid fibroblasts homozygous for the T13H translocation produced only 4 tumours out of a total of 167 mice given inocula of  $2-3\times10^6$  cells per animal<sup>18</sup>. The kinetic constants for two clonal populations of this cross, clones 7 and 8, and a tumour produced from clone 8 (clone 8T1) are shown in Table 3. The hybrids in which malignancy is suppressed show high  $K_m$  values (more than three times the value for the parental PG19 tumour cells); the tumour produced by clone 8 shows a marked reduction in  $K_m$  compared with that of clone 8 itself.

## A melanoma derivative selected for resistance to wheat-germ agglutinin

Bramwell and Harris<sup>6</sup> found that selection of PG19 cells for their ability to grow in lethal concentrations of wheat-germ agglutinin produced derivatives with greatly reduced tumorigenicity. One such line (C) produced no tumours in 17 animals with inocula of  $5 \times 10^4$  cells per animal. This line was recloned and one of the secondary clones, PG19WGAR clone C2, was assayed. This non-tumorigenic clone had a high  $K_m$  comparable with that of the PG19×T13HT13H fibroblast hybrids in which malignancy was suppressed (Table 3). Changes in tumorigenicity produced by wheat-germ agglutinin selection are thus also associated with changes in the  $K_m$  for hexose transport.

### Fibrosarcoma × lymphocyte crosses

Crosses between diploid lymphocytes and the malignant L-cell derivative A9HT show suppression of malignancy  $^{20}$ . Assay of two highly suppressed clones, clones 3 and 4, showed their  $K_{\rm m}$  values to be  $2.240\pm0.160$  and  $2.080\pm0.120$  (Table 4), compared with  $1.170\pm0.079$  for the parental tumour cell (Table 1). A tumour derived from another such clone (A9HT  $\times$  C57BL lymphocyte clone 2T1) resembled the parental tumour cell in having a  $K_{\rm m}$  value of  $1.123\pm0.046$ .

## Fibrosarcoma derivatives selected for resistance to wheat-germ agglutinin

Selection of A9HT cells for resistance to wheat-germ agglutinin produced some derivatives that had lost tumorigenicity and others that retained it<sup>21</sup>. Clones A9HTWC and A9HTWD were both resistant to 25  $\mu g$  ml $^{-1}$  wheat-germ agglutinin, but whereas clone WC failed to produce any tumours with inocula up to  $6.6\times10^6$  cells per animal, clone WD remained as tumorigenic as the original A9HT parent cell. Table 4 shows that the  $K_{\rm m}$  for hexose transport of the non-tumorigenic clone is more than twice that of the tumorigenic clone.

Table 4 Kinetic constants of hexose uptake in fibrosarcoma×lymphocyte hybrids and in lectin-resistant fibrosarcoma derivatives

Cell type	Tumori- genicity	Cell density (cells cm <sup>-2</sup> )	К <sub>т</sub> (mM)	V <sub>max</sub> (nmol per 10 <sup>6</sup> cells per h)
A9HT×C57BL lymphocyte C13	-	2.6×10 <sup>5</sup>	$2.240 \pm 0.160$	247.6 ± 13.2
A9HT × C57BL lymphocyte C14 A9HT × C57BL	*****	$2.5 \times 10^5$	$2.080 \pm 0.120$	451.3 ± 20.8
lymphocyte C12T1	+	$2.0\!\times\!10^{5}$	$1.123 \pm 0.046$	$119.4\pm2.8$
A9HT WC A9HT WD	+	$1.8 \times 10^{5} \\ 1.8 \times 10^{5}$	$\begin{array}{c} 1.685 \pm 0.151 \\ 0.731 \pm 0.087 \end{array}$	$133.9 \pm 7.6$ $120.4 \pm 7.9$

Table 5 Effect of continuous culture in olive on the kinetic parameters of hexose uptake in osteosarcoma×fibroblast hybrida

	-			
H <del>ybri</del> d clone	No. of days m continuous culture	Cell density (cells cm <sup>-2</sup> )	<b>K_</b> (mM)	(nmol per 10 <sup>6</sup> cells per h)
а	3	2,1×10 <sup>5</sup>	$2.986 \pm 0.140$	$445.3 \pm 16.3$
-	7	1.4×10 <sup>3</sup>	$2.499 \pm 0.267$	$243.6 \pm 19.1$
	9	1.6×10 <sup>5</sup>	$1.896 \pm 0.130$	$354.6 \pm 17.2$
	16	2.0×10 <sup>5</sup>	$1.156 \pm 0.108$	$129.5 \pm 9.42$
	22	2.9×10 <sup>3</sup>	1.420±0.376	$462.6 \pm 39.0$
F	6	1.8×10 <sup>5</sup>	3.218 ± 0.529	$372.1 \pm 37.6$
	13	$2.4 \times 10^{5}$	$2.220 \pm 0.052$	$723.0 \pm 11.0$
	40	$6.3 \times 10^4$	$1.363 \pm 0.092$	240.6±7.4

#### Polyoma virus-induced osteosarcoma × fibroblast crosses

As previously described, hybrids between SEWA and diploid cells are highly unstable<sup>18,22</sup>. Malignant segregants are generated in this cross at a very high frequency so that the tumorigenicity of the hybrid clones is variable. Hybrids that initially fail to produce tumours become tumorigenic again on continued cultivation in vitro. Early assay of a fresh set of nine hybrid clones isolated from a cross between SEWA and fibroblasts homozygous for the Rb7BnR translocation showed  $K_m$ values for these clones ranging from 0.8 to 2.9 mM; in some cases the reciprocal plots were initially curvilinear, as described for the YACIR×fibroblast crosses. The clones isolated were obviously a heterogeneous collection. Two clones, C1 and F, which initially gave high Km values, were grown continuously in vitro and their K values determined at intervals. The results (Table 5) show that on continued cultivation the  $K_{-}$  values fell progressively. Clone F, tested in vivo, initially produced no tumours, but soon became tumorigenic. The tumours produced from clones C1 and F gave lower  $K_m$  values than those of the

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cells inoculated. There is clearly further selection for lower  $K_m$ in vivo.

#### Discussion

Our findings leave little doubt that malignancy, as defined by the ability of cells to grow progressively in vivo, is closely linked to a decrease in the  $K_m$  of the membrane hexose transport system. As the difference between malignant and non-malignant cells becomes more pronounced at lower external hexose concentrations, it is not difficult to see how the reduction in  $K_m$  might confer a selective advantage on malignant cells in vivo. The cell density in a primary tumour nodule is high and the blood supply precarious, so that the availability of hexose could easily be limiting. A decrease in the  $K_m$  of the hexose transport system might thus make the malignant cell a more effective scavenger of whatever hexose is available than the normal cells with which it must compete

Two general questions arise concerning the mechanism of the change in  $K_m$ . The first is whether it affects only the hexose transport system or whether other membrane transport systems behave similarly in malignant cells. The second question is whether the change in  $K_m$  reflects the operation of a structurally modified or different hexose transport system, or whether this change is produced as a secondary consequence of more generalized membrane changes. The answer to the latter question must wait until the elements of the hexose transport system have been unequivocally identified and subjected to formal structural analysis. In the light of our previous findings6, we are exploring the possibility that modification of the carbohydrate moiety of the hexose transport protein influences the  $K_m$  of the transport process.

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## Innermost parts of accretion disks are thermally and secularly stable

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There is strong evidence that various types of exotic astronomical objects (quasars, active galactic nuclei, Cyg X-1, SS433) can be explained by accretion disks orbiting black holes. Most of the proposed models of such objects are plagued by instabilities. It is widely held, for example, that all accretion disks must be thermally and secularly unstable in their innermost parts, which seems to be in direct conflict with observations. Much effort has been made to find a stabilizing mechanism operating in the innermost parts of the disks. Here I show that such a mechanism does exist. It is of general relativistic origin, is purely mechanical-operating independently of viscosity and other microphysical processes—and is similar to the mass loss caused by Roche lobe overflow in close binaries.

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It is generally believed that the inner regions of standard, thin accretion disks are both thermally and secularly (L-E) unstable. This is because the most important stability condition,

$$\left. \frac{\mathrm{d} \ln Q^{+}}{\mathrm{d} \ln H} \right|_{U} < \frac{\mathrm{d} \ln Q^{-}}{\mathrm{d} \ln H} \right|_{U} \tag{1}$$

is not satisfied by any one of the possible relevant cooling mechanisms  $(Q^{-})$  when the dissipation, the rate of heat generation  $(Q^+)$ , is determined by the standard viscosity law. In equation (1), H denotes the height of the disk and U the surface density.

Piran<sup>1</sup> gives the most general discussion of the stability problems in terms of four quantities k, l, m, n

$$k = \frac{\mathrm{d} \ln Q^{-}}{\mathrm{d} \ln H} \Big|_{U}, \qquad l = \frac{\mathrm{d} \ln Q^{-}}{\mathrm{d} \ln U} \Big|_{H} \tag{2}$$

$$m = \frac{\mathrm{d} \ln \nu}{\mathrm{d} \ln H} \Big|_{U}, \qquad n = \frac{\mathrm{d} \ln \nu}{\mathrm{d} \ln U} \Big|_{H} \tag{3}$$

which describe phenomenologically a wide range of different viscosity laws and cooling mechanisms, in particular all those discussed in the accretion disk context. In equation (3), the quantity  $\nu$  is the kinematic viscosity. The standard viscosity

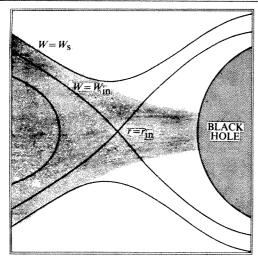


Fig. 1 Equipotential surfaces close to the inner edge of the disk.

law has m = 2, n = 0. Non-standard viscosity laws have m < 2,

A recently discovered<sup>2</sup> mass loss mechanism which operates close to the inner edges of accretion disks orbiting black holes removes both thermal and secular instabilities. There are no instabilities even in the extreme case when the ratio of radiation pressure to the total pressure,  $\beta = 1$ , and when the Shakura-Sunyaev  $\alpha$ -viscosity parameter,  $\alpha = 1$ . The conclusion holds for both standard and non-standard viscosity laws.

Piran's arguments and his stability conditions are used here. After concluding that there is no combination of any known cooling mechanism with the standard viscosity law which gives rise to a stable inner disk region (radiation pressure and electron scattering-dominated), Piran remarks that a secondary wind escaping from the disk surface would have a stabilizing effect. He admits, however, that generally this wind would be too small to change the main conclusion significantly. Nevertheless, he gives explicit forms of the necessary and sufficient stability conditions for thermal and secular stability of a disk with a secondary wind. The conditions are general and valid not only for winds, but for any mass loss from the surface. The mass loss is expressed by

$$\dot{M} \sim U^s H^r \tag{4}$$

and the stability conditions yield

$$(8s-3r)+(51s-9r)\beta-(3s+12r)\beta^2-X^2(m-k)>0$$
 (5a)

$$r(n+1-l)-s(m-k)>0$$
 (5b)

$$[2m+8(n+1)]+[9m+51(n+1)]\beta-3[m+n+1]\beta^2>0$$
 (5c)

$$[sm - (n+1)r] - Y^{2}[ml - (n+1)k] > 0$$
 (5d)

The explicit form of  $X^2$  and  $Y^2$  is not relevant here.

General relativistic effects in the gravitational field of a black hole cause a characteristic behaviour of the equipotential surfaces close to the inner edge of an accretion disk: one of the equipotentials,  $W = W_{\rm in}$ , crosses itself on the edge (see Fig. 1). If the surface of the disk,  $W \approx W_{\rm s}$ , slightly overflows the critical equipotential  $W = W_{in}$ , then mechanical equilibrium is slightly destroyed, causing mass loss. The physical picture is analogous to the case of Roche lobe overflow in close binaries.

Abramowicz, Calvani and Nobili<sup>3</sup> give expressions for the mass loss rate and efficiency of the cooling mechanism connected with the mass loss assuming only mechanical, not thermal, equilibrium. Their equations (5.19) and (5.13) valid close to the inner edge of the disk, that is for  $r \approx r_{\rm in}$ , read

$$\dot{M} \sim UH$$
 (6)

$$O^- \sim UH^3 \tag{7}$$

Thus, for this particular mass loss mechanism s = 1, r = 1, l = 1and k = 3 (for  $r \approx r_{in}$ ).

This result, when combined with the general stability criteria of equation (5), proves that accretion disks orbiting black holes are thermally and secularly stable close to their inner edges. This is true for both standard and non-standard viscosity laws and it does not depend on any particular value of  $\alpha$  or  $\beta$ .

Note that the stabilizing mechanism is purely general relativistic in its origin. This may be the only known situation when a general relativistic effect stabilizes a configuration, especially a rotating one.

The physics beyond this formal proof is simple: consider a small, purely thermal (that is with U = constant) perturbation which produces an excess heat  $(Q^+ > Q^-)$ . If the excess heat cannot be removed from the disk, the disk expands. This causes increased Roche lobe overflow: the location of the Roche lobe depends only on the angular momentum at  $r = r_{in}$  and does not change during the expansion. Because the cooling increases very strongly with the increasing overflow and the heating does not, the disk reaches the state  $Q^- = Q^+$  and achieves thermal stability.

More detailed treatment of this phenomenon will be published elsewhere where I shall discuss the radial size of the region,  $r_{\rm in} \le r \le r_*$ , affected by this stabilizing mechanism. The size, of course, depends strongly on the particular situation. A rough estimate gives  $r_* > H(r_{in}) f(r_*) \approx \text{few } r_{in}$ , where f =(thermal time scale/free-fall time scale). For the supermassiveblack-hole case this is enough to stabilize the whole inner (radiation pressure- and electron scattering-dominated) region of the disk.

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### Milliarcsecond structure of BL Lac during outburst

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The well known object BL Lacerate is the prototype of a class of compact extragalactic sources which display rapid flux and polarization changes at radio and optical wavelengths and a nearly featureless optical spectrum<sup>1</sup>. After a period of relative quiescence, BL Lac has recently undergone a violent outburst at radio wavelengths, accompanied by rapid changes in degree of polarization and position angle (Fig. 1)<sup>2,3</sup>. For a better understanding of the physical mechanism responsible for the variable emission, we have mapped the radio structure at 5 and 10.6 GHz at three epochs during the large flux outburst of 1980 with an intercontinental VLBI array using telescopes in Bonn, West West Germany: Green Bank, Virginia; Westford. Massachusetts; Fort Davis, Texas; and Owens Valley, California. The synthesized beam had a resolution of about 1.0 m arc s at  $\lambda$  6 cm and 0.5 m arc s at  $\lambda$  2.8 cm, corresponding to linear sizes of 5.9 and 2.9 lyr (light year) at the source (using a redshift of 0.0695 and a Hubble constant of 55 km s<sup>-1</sup> Mpc<sup>-1</sup>). We show here that comparison of the size and flux density of the core component with the flux history provides evidence for relativistic beaming effects, independent of detailed model considerations. The flux densities shown in Fig. 1 were obtained at the University of Michigan radio observatory.

The observations were made at three epochs: 1980.41  $(\lambda 6 \text{ cm})$ , 1980.73  $(\lambda 6 \text{ cm})$  and 1980.93  $(\lambda 2.8 \text{ cm})$ . During each epoch, one telescope had a technical failure so that only four telescopes were used in each mapping analysis. The telescopes used the MKII VLBI recording scheme<sup>5</sup> and were equipped

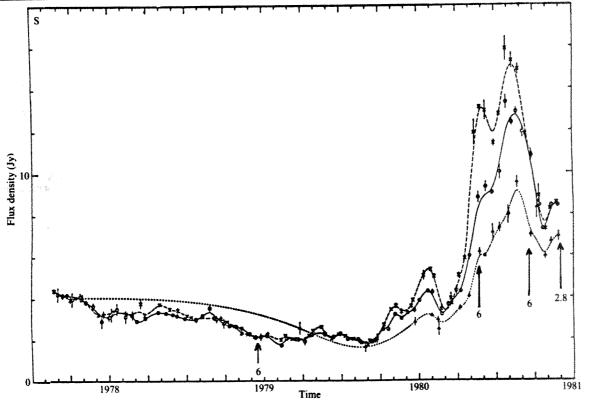


Fig. 1 Flux density compared with time at 4.8 GHz ( $\triangle \cdots \triangle \cdots$ ), 8.0 GHz ( $\longrightarrow$ ) and 14.5 GHz ( $\times -- \times --$ ). Data points are 2-week averages. Epochs of VLBI observations are shown by arrows where the number indicates wavelength of each observations.

with maser time standards. All observations were made using left circular polarization and a receiving bandwidth of 2 MHz. The data were correlated using the Caltech/JPL VLBI correlator. The mapping was done at the University of Iowa using a hybrid algorithm utilizing closure phase. For all three epochs, the resulting fits to the data were very good, and the maps are thought to be reliable to the 5% contour level. An example of the visibility data and corresponding hybrid model fit is given in Fig. 2 for the 1980.93 data at  $\lambda$  2.8-cm wavelength.

The  $\lambda$  2.8-cm hybrid map is shown in Fig. 3a, while the two  $\lambda$ 6-cm maps are shown in Fig. 4a, b. In each case, the restoring beam has been chosen by fitting an elliptical gaussian to the main beam of the array power pattern, which is obtained by Fourier transforming the transfer function for each observation. A remarkable aspect of the structure at all three epochs which is not apparent on the hybrid maps is the extreme narrowness of the source. For both  $\lambda$  6-cm observations, the source was completely unresolved when the baselines were aligned approximately east-west. The  $\lambda 2.8$ -cm data also show this effect, although there is some east-west resolution on the transatlantic baselines. This dominant north-south structure has been seen with more limited VLBI measurements<sup>7-9</sup> as early as 1971 and apparently indicates that there is an alignment mechanism in the central source which is stable compared with the time scale of individual flux outbursts.

To clarify the detailed north-south structure, we have shown one-dimensional profiles of brightness temperature along a position angle of  $0^{\circ}$  for each map. Figure 2b shows the  $\lambda$  2.8-cm profile, while Fig. 5 shows the  $\lambda$ 6-cm profiles at both epochs. In addition, we have shown the north-south brightness profile for the three-component gaussian model of Pearson and Readhead based on data taken at  $\lambda$ 6-cm in 1978 December when BL Lac was dormant (see Fig. 1). To compare brightness temperatures in a uniform way, we have used the same restoring beam for all three  $\lambda$ 6-cm profiles. This beam is too small for the December 1978 model (because only US baselines were used) so that the detailed structure of the components is probably unreliable. We have also convolved our data with the same restoring beam of Pearson and Readhead and find no evidence

of the southerly component extending  $\sim 10 \text{ m arcs s } (60 \text{ lyr})$  seen in their 1978 map.

Comparison of the  $\lambda$  2.8 map (Fig. 3) with the flux history data of Fig. 1 shows that the source is much larger than would be expected from simple light travel time arguments. The current outburst can be dated from ~1979.8 when the total flux density is  $\sim 2$  Jy at  $\lambda$  6 cm and  $\lambda$  2.8 cm. Without relativistic effects, the maximum source size in ~1980.9 would be 1.1 lyr, whereas the north-south extent of the  $\lambda$  2.8-cm map is  $\sim 4.5$  lyr. This discrepancy could be understood in several ways: (1) simultaneous, independent brightening of causally unconnected regions; (2) activation by a single 'trigger' mechanism located approximately midway between two distant emitters; or (3) relativistic beaming effects causing a time 'speed-up' in the observed flux changes. Explanation (1) seems very improbable, particularly considering the 3-yr dormant period which preceded the outburst, while explanation (2) requires a highly specialized geometry.

The relativistic beam model<sup>11</sup> has been widely used to explain the properties of compact radio sources<sup>12-14</sup>. The model consists of a central object with two opposed particle beams moving with relativistic bulk velocities. If the line of sight to the observer is nearly parallel to the beam axis, he will see the forward beam Doppler-boosted in intensity. The true angular source size is the apparent size as observed in the VLBI data, but the flux variations are observed 'speeded up' by the Lorentz factor, which in the present case is  $\gamma \sim 4$ .

A further argument supporting relativistic beaming in this source is the anomalously high brightness temperatures implied by the flux density variations. In the absence of relativistic effects, the inferred brightness temperature of the core component would be at least  $10^{13}$  K based on the light travel size. This violates the inverse Compton limit of  $T_{\rm B} \leq 10^{12}$  K for an opaque synchrotron emitter. On the other hand, the observed brightness temperatures (based on the actual measured angular sizes) are  $T_{\rm B} \leq 3 \times 10^{11}$  K for all three observations.

The  $\lambda$  6-cm maps do not have sufficient angular resolution to show the details of the evolution of the jet during the outburst. However, comparison of the 1980.41 and 1980.73 brightness

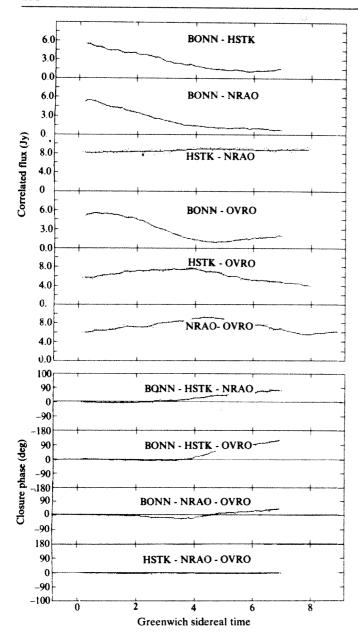


Fig. 2 Four-min averages of fringe amplitude and closure phase for the 1980.93 data at 10.6 GHz. Also shown is the fit from the hybrid map shown in Fig. 3a.

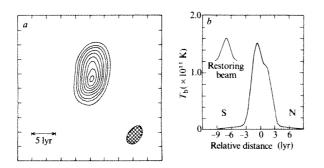
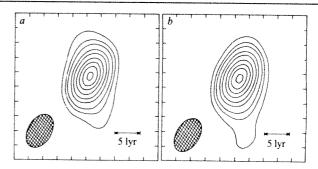


Fig. 3 a, Hybrid map of BL Lac at 10.6 GHz, epoch 1980.93. Contours are 5, 15, 25, 35, 45, 55, 65, 75, 85 and 95% of peak flux. b, One-dimensional profile of peak brightness temperature along position angle 0° in map in a.



a, Hybrid map of BL Lac at 5 GHz, epoch 1980.41. Contours as in Fig. 3a. b, Epoch 1980.73, same as in a.

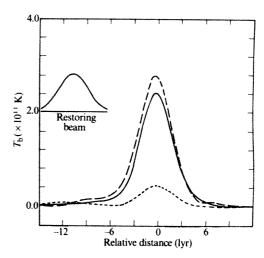


Fig. 5 One dimensional profiles of peak brightness temperature along position angle 0° for 5 GHz maps at three epochs. Dotted line is epoch 1978.93, solid line is epoch 1980.41 and dashed line is epoch 1980.73. Note that the relative position between epochs can not be determined from the VLBI data and is not necessarily as shown here. Also the same restoring beam has been used for all three epochs. This over-resolves the 1978.93 data (see text).

profiles (Fig. 5) show that there has been a small, but definite increase in the core size, of  $\sim 0.5$  lyr. This is actually a lower limit, as the true size has been convolved with a restoring beam whose dimensions are comparable with the core size. In addition, all three profiles show a weak, extended southerly component. The detailed nature of this component is uncertain because of the limited dynamic range of the maps. However, we have found differences in the closure phase on identical baseline triangles in the comparison of 1980.41 and 1980.72 data which we are confident are caused by changes in the extended southerly structure. The extended structure is not seen on the  $\lambda$ 2.8-cm profile, indicating that perhaps the extended emission has become optically thin between  $\lambda$  6 and  $\lambda$  2.8 cm.

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# Diffuse interstellar absorption bands between 2.9 and 4.0 µm

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IRS7, the obscured M supergiant near the galactic centre, is an excellent source against which to study interstellar absorption bands near 3- $\mu$ m wavelength. Previously we considered the 3.4- $\mu$ m absorption band as evidence that interstellar grains include an organic component. We report here superior data between 2.9 and 4.0  $\mu$ m, and find a broad absorption trough with numerous discrete features stretching from 2.9 to 3.6  $\mu$ m. Although no definite identification of the material producing this absorption is possible, there is some indication that complex organic molecules may be involved.

Because of the strong and variable telluric absorption features in the waveband of interest, we now describe our observing methods in some detail. We used the IR photometer-spectrometer at the f/15 focus of the 3.9-m Anglo-Australian Telescope. In this instrument a circular variable filter (CVF) is scanned rapidly and repeatedly across the image of a source; in this case each spectral scan between 2.9 and 4.0 µm required 5.8 s. The resolution of the CVF is  $\lambda/\Delta\lambda \sim 100$ . Scans were alternated in direction, and at the end of each pair of scans the telescope was driven back and forth between IRS7 and a patch of sky 80 arcs to the north. This procedure ensured that the data were not contaminated by signals from other sources in the galactic centre area appearing in the reference sky beam. A circular aperture of 3.5 arcs diameter was used to reject nearby sources, in particular the very cool object IRS3. The resulting spectrum shows a weaker contribution from cool dust at 4 µm than any previously published. Indeed, IRS7 may have no intrinsic excess radiation from circumstellar dust, and the contribution we see may be contamination from nearby sources.

Approximately every 5 min we made observations in exactly the same manner of the nearby K0 giant star  $\gamma$  Sgr. Five separate observations of IRS7 were individually bracketed by observations of  $\gamma$  Sgr. All observations were made when both objects lay within  $10^{\circ}$  of the zenith. The observations of  $\gamma$  Sgr monitored changes in the telluric absorption bands. The changes found on this occasion were quite small.

Figure 1 shows our raw data in the vicinity of the strongest telluric features, between 2.9 and 3.45  $\mu$ m. Figure 1b refers to the ratio IRS7/ $\gamma$  Sgr, and shows the five separate observations, displaced for clarity, and their mean: the major features repeat well from one spectrum to the next. Note the narrow absorptions near the channels 23 and 30. We present the raw spectra of  $\gamma$  Sgr, showing the telluric absorptions in Fig. 1a. These are displaced from the features in the quotient spectra, indicating that the latter do not arise from poor cancellation of the telluric features

An independent observation made in July 1980 enables a final check of these features. This observation was less favourable because IRS7 and  $\gamma$  Sgr lay at a greater zenith distance and a different CVF gave a resolution of only 50. Finally, the 1980 data used a larger aperture and smaller beam separation. Nonetheless, between 2.9 and 3.45  $\mu$ m the same spectral features were seen except for the first few data points at the short-wavelength end of the spectrum.

Also shown in Fig. 1a is the ratio of the spectrum of  $\gamma$  Sgr to a G dwarf star, whose spectrum is expected to be featureless in this spectral region. The telluric cancellation is not as good on this occasion because of the greater time gap between the two observations. This ratio indicates that the spectral features

observed in IRS7 are not introduced by the use of  $\gamma$  Sgr as a calibrator

Spectra obtained of the M supergiant VX Sgr confirm that it does not show any absorption features in the 2.9–4.0  $\mu$ m region. Thus all the absorption bands in the spectrum of IRS7 are of interstellar origin.

Figure 2 shows the resultant spectrum, including the portion from 2.0 to 2.5  $\mu m$ . The deep photospheric CO absorption of the M supergiant is clearly seen in this spectral region, and as expected there is no indication of the 1.9 or 2.7  $\mu m$  steam absorption bands. Outside the CO band we expect IRS7 to show a black-body continuum. Note that there is roughly a 10% uncertainty in the relative heights of the 2 and 3  $\mu m$  spectra caused by the necessary use of a small aperture and the presence of seeing of 1–2 arc s. The broken line on Fig. 2 indicates where the 1980 spectrum digresses from the 1981 data.

To reveal the depth of the absorption we have constructed a continuum in Fig. 2 which we have forced to fit through the 2.0-2.25 and 3.6-3.95 µm sections of the data. Three parameters describe such a continuum: the black-body temperature of the star, assumed to be 3,200 K, the extinction at a chosen wavelength (2.2 µm: 2.5, derived from ref. 1), and the form of the reddening law. For the latter we use  $A_{\lambda} \propto \lambda^{-n}$ , where  $A_{\lambda}$  is the reddening in stellar magnitudes at wavelength  $\lambda$ . We assumed n = 2 based on ref. 3. With any reasonable adjustment of these parameters we cannot fit the designated continuum points. To do so we add a cool black-body component representing emission from dust. This may arise in IRS7 itself, in the nearby IRS3, or in fainter, unmapped sources. Two more parameters are then involved: the dust temperature and the intensity of the dust emission. The continuum plotted in Fig. 2 has a dust component of 550 K having 0.19 of the star's flux at

Relative to this continuum we find a complex of absorption

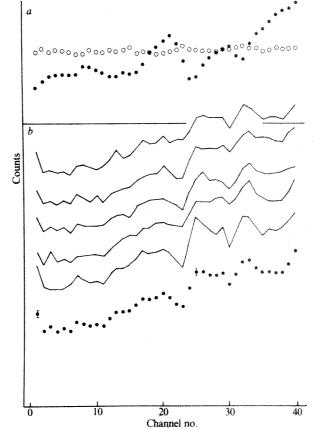


Fig. 1 b, IRS7 divided by the standard  $\gamma$  Sgr plotted against channel number in the region of the telluric bands  $(2.9-3.45 \,\mu\text{m})$ . The solid curves are based on five individual sets of observations obtained as described in the text.  $\bullet$ , The mean spectrum a, The raw spectrum of  $\gamma$  Sgr showing the telluric bands  $(\bullet)$  and the ratio of  $\gamma$  Sgr (K0) to that of a G dwarf star  $(\bigcirc)$ .

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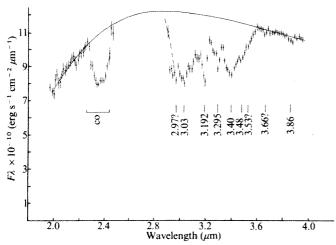


Fig. 2 The 2.0-4.0 μm spectrum of IRS7. The solid curve is the estimated position of the continuum (see text). The dashed curve is the July 1980 spectrum where it deviates from the May 1981 spectrum.

bands stretching from 2.9 to 3.6 µm. Two dominant bands lie at 3.03 and 3.40  $\mu$ m, and have optical depths of 0.41 and 0.32 respectively. The increased optical depth of the 3.4 µm feature relative to our previous result1 reflects the improved definition of the continuum. This optical depth would be further increased if the redness between 3.6 and 3.95 µm were due to absorption rather than to the addition of cool dust.

Figure 2 shows all of the absorption features we believe to be real, and a few (marked?) which are less certain. The absorption clearly breaks up into many discrete features and two of these (at 3.192 and 3.295 µm) are quite narrow. Whilst the broad features centred at 3.03 µm and 3.40 µm presumably arise in solid grains, the narrow features may have a gaseous origin. We have found no convincing identification for either, although many hydrocarbons have stretching absorptions in this spectral range. However, laboratory data are not available for even quite simple, incompletely bonded molecules (for example CH<sub>3</sub>) such as might be found in the interstellar environment. Quite narrow absorption bands are found in the absorption spectra of some solids, such as methane<sup>4</sup> and methyl alcohol<sup>5</sup>.

Suitable data on the absorption properties of solid materials are not available, and laboratory work is needed to match the present spectra. We see no evidence for water ice in the available data. The absorption band at 3.03 µm is displaced from that of water (3.06 µm) and is narrower. If water ice is present, it contributes little to the absorption. Similarly, solid ammonia (2.91 µm) is not present. Molecules involving carbon and hydrogen, on the other hand, can produce absorption near both 3.0 and 3.4 µm, and are thus particularly attractive identifications. Organic molecules containing OH and NH bonds also cause absorption near 3.0 µm (refs 6, 7). The wavelengths of CH, NH and OH vibrations depend critically on the composition of the solid and only a few cases<sup>5</sup> of astronomical interest have been discussed in the literature. The H bonded solid complex of H<sub>2</sub>O, CH<sub>3</sub>OH and NH<sub>3</sub> (ref. 5) has a feature due to NH at 2.97 µm but there is no correspondence with the rest of the spectrum.

It has been suggested recently<sup>8</sup>, that surface functional groups attached to reactive sites on small carbon grains may be responsible for the IR features seen in IRS7. Although there are some interesting wavelength coincidences (aromatic -CH (3.3 \mum),  $CH_3$  (3.4, 3.5  $\mu$ m), -CHO (3.5, 3.65  $\mu$ m)) no information has been given on band strengths and shapes to enable a detailed comparison.

We have looked at published spectra of specific grain models involving organic polymers which could be possibilities for the organic component of interstellar grains. The polymer-like material in carbonaceous chondrites show absorption due to CH near 3.3  $\mu m$  and weak absorption at 3.0  $\mu m$ . Clearly this material will not produce a fit to the astronomical data even over a restricted wavelength region. However, hydrated silicates of

meteoritic origin show a broad absorption feature centred near 3 μm<sup>10</sup>. This may be relevant in view of the possible identifications of the 9.7 µm and 18 µm features in the galactic centre with silicates. We consider it significant that the yellow component of UV tholin<sup>2,9</sup> looks qualitatively similar to that of IRS7 in the 3.4-μm region. In particular the shoulder at 3.36 µm, the central component at 3.4 µm and the component at 3.48 µm are present in the laboratory data with wavelengths agreeing to within  $\pm 0.02 \,\mu m$ . This could indicate the existence of complex organic molecules in grains<sup>2,11,12</sup>. However, this material does not produce significant absorption near 3 µm as required by the IRS7 data. It is interesting that the satellite features at 3.36, 3.48 µm are also seen in the spectrum of polyformaldehyde suggesting that H<sub>2</sub>CO may be present as a structural unit13

We cannot rule out the possibility that a mix of simple organics might match the observed spectrum. This requires further investigation using laboratory data. However, as our data are of relevance mainly to the properties of dust in the diffuse interstellar medium it will be more appropriate to look at refractory materials related to the UV component of tholins such as the non-volatile residue produced in the laboratory experiments designed to simulate conditions in the interstellar medium described by Greenberg<sup>12</sup> and with predictions from the Hoyle-Wickramasinghe model<sup>11</sup>. The latter comparison has shown a remarkable similarity between the spectrum of IRS7 and that of dried bacteria (Escherichia coli) which will be reported elsewhere14

Finally emission features are found in this waveband in some H II regions and carbon-rich planetary nebulae (for example, NGC7027)15. These normally comprise a strong, narrow peak at  $3.29 \mu m$  and a weaker, broader feature peaking at  $3.40 \mu m$ . In this respect they mimic features in the relevant portion of the absorption spectrum of IRS7. However, the 3.03 and 3.192 μm features are not seen in emission in any objects. An interpretation of the absorption spectrum of IRS7 which simultaneously predicted the more restricted range of the emission bands would be very satisfying.

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### Fractal dimensions of landscapes and other environmental data

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Mandelbrot has introduced the term 'fractal' specifically for temporal or spatial phenomena that are continuous but not differentiable, and that exhibit partial correlations over many scales. The term fractal strictly defined refers to a series in which the Hausdorf-Besicovitch dimension exceeds the topological dimension. A continuous series, such as a polynomial, is differentiable because it can be split up into an infinite number of absolutely smooth straight lines. A non-differentiable continuous series cannot be so resolved. Every attempt to split it up into smaller parts results in the resolution of still more structure or

Table 1 Estimated D values for various environmental series

Location	Property	Lag	$D$ as $lag \rightarrow 0$	D at max. slope	Ref.
Wales	Soil—sodium content	15.2 m	1.7-1.9*	<del></del>	7
	—stone content	15.2 m	1.1-1.8*		7
	(both over four directions)			-	
England	Soil—thickness of cover loam	20 m	1.6*	A	7
England	Soil—electrical resistivity (4 directions)	1 m	1.4-1.6*		7
England	Surface of airport runway	30 cm	1.5†		8
Descrits in Africa	Soil - mean cone index	~1 km	1.9‡		9 9
and America	-silt + clay in 0-15 cm layer	~1 km	1.8‡		9
	-mean diameter of surface stones	~1 km	1.8‡		9
	-coarse sand fraction in 0-15 cm layer	~1 km	1.8‡		9 9 9 2
	Vegetation cover	~1 km	1.6‡		9
South Africa	Gold	Various	1.9₩		
Australia	Soil—phosphorus level	5 m	2.0‡		10
	— <i>p</i> H	5 m	1.5‡		10
	—potassium level	5 m	1.6‡	1.1‡	10
	-bulk density	5 m	1.5‡		10
1	-0.1 bar water	5 m	1.5‡		10
France	Iron ore in rocks				
	-chlorite	15 µm	1.6*		11
	—quartz	15 µm	1.9*		11
	—quartz	5 cm	1.6*		11
	—iron	5 cm	1.5*		11
	-iron (E-W)	100 m	1.7₩		11
	ron (N-S)	100 m	1.8*	-	11
	iron (E-W)	500 m	1.6*		11
	iron (N-S)	500 m	1.9*		11
France	Sea anemones	10 cm	1.6		12
Chad	Rainfall	1 km	1.7*		13
Mauritania	Iron ore	3 m	1.4*		2
vory Coast	Groundwater levels		•		
٧	Piezometer 1	1 day	1.6*		2 2
	.2	1 day	1.7*		2
	3 .	1 day	1.8*	1.3*	2
	4	1 day	1.3*	1.1*	2
Canada	Oil grades	60 cm	1.7*		2 2 2
Chile	Copper grades	2 m	1.7*	n-ma	2
France	Topographic heights	10 m	1.5*	1.1*	2
JSA	Soil—sand content	10 m	1.6-1.8*		14
•	—pH	10 m	2.0*		14
Worldwide	Crop yields	1-1,000 m	1.6-1.8‡	-	15
India	Water table depth	250 m	1.6*		16

<sup>\*</sup> Estimated from variogram. ‡ Estimated from block variance. † Estimated from power spectrum. § Estimated from covariance.

roughness. For a linear fractal function, the Hausdorf-Besicovitch dimension D may vary between 1 (completely differentiable) and 2 (so rough and irregular that it effectively takes up the whole of a two-dimensional topological space). For surfaces, the corresponding range for D lies between 2 (absolutely smooth) and 3 (infinitely crampled). Because the degree of roughness of spatial data is important when trying to make interpolations from point data such as by least-squares fitting or kriging<sup>2</sup>, it is worth examining them beforehand to see if the data contain evidence of variation over different scales, and how important these scales might be. Mandelbrot's work1 suggests that the fractal dimensions of coastlines and other linear natural phenomena are of the order of D = 1.2-1.3, implying that long range effects dominate. I show here that published data on many environmental variables suggest that not only are they fractals, but that they may have a wide range of fractal dimensions, including values that imply that interpolation mapping may not be appropriate in certain cases.

Berry and Lewis<sup>3</sup> have shown that the Weierstrass-Mandelbrot fractal function (WMF)

$$W(t) = \sum_{n=-\infty}^{\infty} \frac{\left[ (1-\mathrm{e}^{\mathrm{i}\gamma^n t}) \, \mathrm{e}^{\mathrm{i}\phi_n} \right]}{\gamma^{(2-D)n}}$$

 $(1 < D < 2, \gamma > 1, \phi_n = arbitrary phases)$ 

has a power spectrum  $P(\omega)$  that varies approximately as  $\omega^{-(5-2D)}$ , and a variance of increments  $V(t) = ([W(t_0) - W(t_0 + t)]^2)$  that varies as  $t^{A-2D}$  at the origin. If D > 1.5, V(t) is itself a fractal function. These results allow us to

estimate the fractional dimension D of a real series, either from the slope of the log-log plot of the power spectrum as  $t \to 0$ , or from

$$\frac{d \log V(t)}{d \log t} = 4 - 2D \qquad (t \to 0)$$

The variance of increments (or the half thereof, the semi-variance) is much used in geostatistical studies where, computed over distances, it is referred to as the variogram<sup>2</sup>. Computing the variogram is the first step in the interpolation procedure known as kriging which is used to assist estimation of mineral reserves, contouring groundwater surfaces, and so on. Thus many published data are available in this form and it is then a simple matter to calculate their dimensions D relative to the sampling interval used.

For a second-order stationary series, the variance of increments at a given lag is equal to twice the difference between the variance of the series and the covariance. Thus, D values may also be computed from covariances. In agriculture and soil science, many data have been published in the form of block variances; that is the variance within blocks of equal size plotted against block size. Because Yates showed that the variance for a block of a given size k is

$$s_k^2 = \frac{2}{k(k+1)} \sum_{h=1}^k (k-h+1) Vh$$

where  $s_k^2$  is the block variance of block length k, and Vh the variance increment for lag h, the slope of log Vh versus  $\log h(h \to 0)$  can also be estimated from these data.

Table 1 presents a selection of data so analysed, giving the study location, the type of environmental variable, the lag interval used for sampling and the estimated D value assuming that the real data are but a series of regularly spaced samples of a realization of the Weierstrass-Mandelbrot function over onedimensional space or time.

The data support Mandelbrot's assertion that D values of landscape and other data may range over many values. It is evident that most of the values reported here exceed 1.5, and many are greater than 1.8. Note that one of the smoothest surfaces imaginable in a landscape, a new airport runway, has a relatively high D, presumably because variations over long distance are low in amplitude. These data do not conform to a single roughness model as proposed by Sayles and Thomas,5 indeed, as Berry and Hannay<sup>6</sup> have commented, much wider ranges of roughness or randomness are to be expected.

These results should not be accepted uncritically, however. First, it is important to realize that the Weierstrass-Mandelbrot function is just one of a class of 'model' fractals. Its peculiarity is that it has a discrete and geometric spectrum, and this might make some of its properties non-universal among other fractals with the same D (M. V. Berry, personal communication).

Second, although some environmental data do appear to display the fractal property of statistical self-similarity at all scales, there are also many that show self-similarity over a limited range of scales, or over a few widely separated scales. For example, a variable with a highly regular spatial variability has a variogram that exhibits parabolic behaviour near the origin<sup>2</sup>. This is a good example where d log  $V(h)/d \log h$  becomes less steep as  $h \to 0$ , and it indicates that the ultra-short, short and middle range variations are trivial compared with the main variations seen at larger scales. In fact, computing D as  $h \to 0$  will in this case estimate D for the ultra-short range scales of variation.

Third, the form of the variogram is often highly dependent on sampling direction and sampling interval. It is well known that when a particular sample spacing tends to match the scale of a spatial pattern, the variance of increments can fall rapidly. Accordingly, when a sampling interval matches a particular scale of a phenomenon in the landscape it perceives an apparently lower D. If this is so, it would seem more appropriate to estimate D values from the parts of the variogram showing maximum slope. Table 1 also contains these data for those phenomena showing a maximum slope at positions other than

The results presented here suggest that Mandelbrot's D can be used as a useful indicator of the complexity of autocorrelations over many scales for natural phenomena. However, although many natural phenomena do display certain degrees of statistical self-similarity over many spatial scales, there are others that seem to be structured and have their levels of variability clustered at particular scales. This behaviour does not exclude them from the fractal concept. Mandelbrot<sup>1</sup> considers that it is quite acceptable to have a series of zones of distinct dimensions connected by transition zones. If this is reasonable, it means that the examination of D values would be useful for trying to separate scales of variation that might be the result of particular natural processes. Moreover, identifying such scales could be of enormous practical value because one could then tailor sampling to a particular scale range of the phenomenon in question, thereby improving the efficiency of expensive field investigations and the resulting interpolations. The high level of the D values for some soil and geological data reported here would seem to question the wisdom of interpolation mapping in certain instances, however, and it would seem worthwhile to use D values as a guide to how further mapping and interpolation should proceed.

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### Reactor-released radionuclides in Susquehanna River sediments

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Three Mile Island (TMI) and Peach Bottom (PB) reactors have introduced <sup>137</sup>Cs, <sup>134</sup>Cs, <sup>60</sup>Co, <sup>58</sup>Co and several other anthropogenic radionuclides into the lower Susquehanna River. Here we present the release history for these nuclides (Table 1) and radionuclide concentration data (Table 2) for sediment samples collected in the river and upper portions of the Chesapeake Bay (Fig. 1) within a few months after the 28 March 1979 loss-of-coolant-water problem at TMI. Although we found no evidence for nuclides characteristic of a ruptured fuel element, we did find nuclides characteristic of routine operations. Despite the TMI incident, more than 95% of the total 134Cs input to the Susquehanna has been a result of controlled low-level releases from the PB site. 134Cs activity released into the river is effectively trapped by sediments with the major zones of reactor-nuclide accumulation behind Conowingo Dam and in the upper portions of Chesapeake Bay. The reported distributions document the fate of reactor-released radionuclides and their extent of environmental contamination in the Susquehanna-Upper Chesapeake Bay System.

During the past 14 yr, five nuclear power reactors have operated for varying lengths of time at two sites on the Susquehanna River (Fig. 1). The first reactor, PB 1, was a 40-MW, gas-cooled reactor and operated from March 1966 to October 1974. Three others, PB units 2 and 3 (boiling-water reactors), and TMI unit 1 (a pressurized-water reactor), began power production in 1974 and each produces ~1,000 MW. TMI unit 2, a twin of TMI 1, was activated on 30 December 1978 but shut down on 28 March 1979 after the loss-of-coolant incident. As with the other nuclear power stations, minor amounts of <sup>137</sup>Cs, <sup>134</sup>Cs, <sup>60</sup>Co, <sup>58</sup>Co and other radionuclides are released with the coolant-water effluent. The radionuclide release history of these reactors has been compiled using Nuclear Regulatory Commission documents1 and is summarized in Table 1. The Peach Bottom plant has contributed most of the reactor-produced radionuclides introduced into the Susquehanna River, and from 1975 to 1979, the PB reactors have released >95% of the total <sup>134</sup>Cs input to the river.

In addition to reactor releases  $^{137}$ Cs (half life of  $\sim 30$  yr) has been introduced into the Susquehanna-Chesapeake Bay system as global fallout from atmospheric nuclear weapons testing. The major influx of fallout <sup>137</sup>Cs occurred between 1962 and 1964. Reil<sup>2</sup> used the distribution of fallout <sup>137</sup>Cs in Chesapeake Bay to study the hydrography of the estuary. Although other fission products and neutron activation nuclides, such as <sup>134</sup>Cs, <sup>66</sup> and 58Co are also produced during weapons tests, their short half lives ( $\sim$ 2, 5 and 0.2 yr, respectively) and low yields cause the

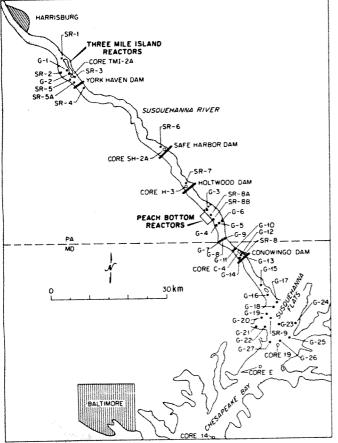


Fig. 1 Location map for sediment samples collected in the Susquehanna River and upper portions of the Chesapeake Bay. Samples labelled SR are surface sediment grabs collected in May 1979 and those labelled G are grabs collected in September 1979.

O, Core samples.

fallout contribution of these nuclides to be negligible. Consequently, observed <sup>134</sup>Cs, <sup>60</sup>Co and <sup>58</sup>Co activities in Susquehanna River sediments are a result of reactor releases only. Previous studies in the River Esk<sup>3,4</sup>, Hudson Estuary<sup>5-7</sup>, Columbia River Estuary<sup>8,9</sup>, James River Estuary<sup>10</sup> and Chesapeake Bay<sup>11-13</sup> have shown the usefulness of these particle-associated radionuclides as tracers for sediment transport and accumulation patterns and as tools for understanding river estuarine sedimentary processes.

Radionuclide activities were measured in surface sediment samples collected from the Susquehanna River and Upper Chesapeake Bay in May and September 1979. The sediment area sampled by each grab was  $\sim 15 \times 20$  cm and the sample depth  $\sim 10$  cm. The sediments were dried, ground with a mortar and pestle, and sealed in  $100\text{-cm}^3$  aluminium cans or  $550\text{-cm}^3$  Marinelli beakers depending on the sample size. The activities of  $^{137}\text{Cs}$ ,  $^{134}\text{Cs}$ ,  $^{60}\text{Co}$ ,  $^{58}\text{Co}$  and  $^{40}\text{K}$  (a naturally occurring radionuclide) were measured using a lithium-drifted germanium detector and multichannel analyser. The activity of naturally occurring  $^{40}\text{K}$  reflects the potassium content of the sediment (0.012% of the potassium in K-bearing minerals is  $^{40}\text{K}$ ) and correspondingly, quartz-rich sandy sediments or organic-rich peaty sediments are characterized by low  $^{40}\text{K}$  activities. Bomb- and reactor-produced  $^{137}\text{Cs}$  was detected in all surface

Bomb- and reactor-produced <sup>137</sup>Cs was detected in all surface sediment samples and activities ranged from 70 to 2,500 pCi kg<sup>-1</sup> (Table 2). The highest activities of reactor-produced <sup>58</sup>Co (a few hundred pCi kg<sup>-1</sup>) were detected in sediment samples near the TMI reactors. Although measurable activities (100–200 pCi kg<sup>-1</sup>) of reactor produced <sup>134</sup>Cs were also observed in the sediments near the TMI reactors (Table 2), the highest <sup>134</sup>Cs activities (400–1,680 pCi kg<sup>-1</sup>) were observed in the sediments near the PB reactors and just downstream behind Conowingo Dam (Fig. 2). We found no evidence in the river sediments for γ-emitting radionuclides which would

Table 1 Annual releases of radionuclides in the liquid effluent from Three Mile Island and Peach Bottom reactor sites

<sup>134</sup> Cs	<sup>137</sup> Cs	<sup>60</sup> Co	<sup>58</sup> Co
(Ci)	(Ci)	(Ci)	(Ci)
and			
< 0.001	0.00\$	0.004	0.052
0.006	0.012	0.006	0.066
0.058	0.075	0.005	0.080
0.145	0.019	0.016	0.481
0.025	0.062	0.018	0.095
0.23	0.15	0.05	0.77
< 0.001	0.006	0.018	0.048
0.913	0.830	0.029	0.010
1.370	1.290	0.125	0.010
2.860	0.810	0.154	0.027
3.92	3.26	0.167	0.024
9.06	6.20	0.49	0.12
	(Ci) and <0.001 0.006 0.058 0.145 0.025 0.23 <0.001 0.913 1.370 2.860 3.92	(Ci) (Ci)  and  <0.001 0.008 0.006 0.012 0.058 0.075 0.145 0.019 0.025 0.062 0.23 0.15  <0.001 0.006 0.913 0.830 1.370 1.290 2.860 0.810 3.92 3.26	(Ci) (Ci) (Ci) (Ci)  and  <0.001  0.008  0.004 0.006  0.012  0.006 0.058  0.075  0.005 0.145  0.019  0.016 0.025  0.062  0.018 0.23  0.15  0.05  <0.001  0.006  0.018 0.913  0.830  0.029 1.370  1.290  0.125 2.860  0.810  0.154 3.92  3.26  0.167

Effluent release data reports from US Nuclear Reglatory Commission documents.

characterize a rupture of the fuel elements (such as  $^{95}Zr^{-95}Nb$ ,  $^{140}Ba^{-140}La$ ,  $^{141}Ce$ ,  $^{144}Ce^{-144}Pr$ ,  $^{108}Ru$  and  $^{106}Ru^{-106}Rh$ ). Table 2 shows that the gamma activities of reactor-released radionuclides in the sediments of the Susquehanna-Chesapeake Bay System are one to two others of magnitude less that that of naturally occurring  $^{40}K$  (Table 2). Concentrations of naturally occurring uranium and thorium decay-series radionuclides also greatly exceed the reactor products in our samples.

Vertical distributions of radiocæsium in our sediment cores are illustrated in Fig. 3. Cores \$H-2A, H-3 and C-4 were collected in May 1979 from behind the Safe Harbour, Holtwood and Conowingo Dams respectively (Fig. 1), and each was ~80 cm long. Box Cores 14 and 19 (each ~40 cm long) were collected in Upper Chesapeake Bay in August 1979. Gravity Core E was taken within the turbidity zone of Upper Chesapeake Bay (Fig. 1) in October 1976.

Although measurable activities of <sup>137</sup>Cs were observed throughout the entire lengths of cores H-3, C-4, E and 14, the activity of reactor-produced <sup>134</sup>Cs was generally confined within the top 10–15 cm (Fig. 3). The deepest appearance of <sup>137</sup>Cs in the sediment core indicates the beginning of significant fallout in

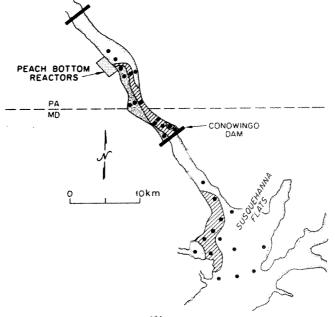


Fig. 2 Areal distribution of  $^{134}$ Cs activity in the surface sediments downstream of the Peach Bottom reactor site. All the samples, including those taken in sandy areas of Upper Chesapeake Bay, contained detectable activities of  $^{134}$ Cs (Table 2). The surface sediment  $^{134}$ Cs activities are kg $^{-1}$ :  $\boxtimes$ , >400 pCi kg $^{-1}$ ;  $\boxtimes$ , 150–400 pCi kg $^{-1}$ ;  $\square$ , <150-pCi kg $^{-1}$ .

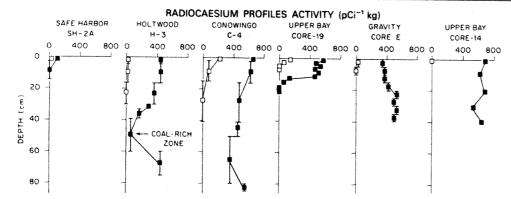


Fig. 3 Vertical distributions of radiocaesium in sediment cores indicate that the areas behind Holtwood and Conowingo Dams and areas within the turbidity zone of Upper Chesapeake Bay are sites of rapid sediment and radionuclide accumulation. Statistical counting errors are approximately the same size as the symbol (□, <sup>134</sup>Cs; ■, <sup>137</sup>Cs) illustrating the data point.

1954, and the deepest appearance of <sup>134</sup>Cs indicates the beginning of significant releases from the TMI and PB reactors. In addition, peaks in the radionuclide profiles may be correlated with the history of fallout or reactor releases to provide additional time-stratigraphical reference levels. Any rigorous attempt at obtaining sediment accumulation rates using these time-stratigraphical markers requires information concerning variations in sediment properties (such as grain size) and the extent of sediment mixing.

Table 2         Reactor-released radionuclides in Susquehanna River surface sediments										
	<sup>137</sup> Cs	134Cs*	60Co*	58Co*	<sup>40</sup> <b>K</b> †					
Location	(pCi kg <sup>-1</sup> )	(pCi kg <sup>-1</sup> )	(pCi kg <sup>-1</sup> )	(pCi kg <sup>-1</sup> )	(pCi kg <sup>-1</sup> )					
Three Mile	: Island									
SR-1	$170 \pm 9$	$-11 \pm 8$	$3\pm7$	$-3 \pm 5$	$7.010 \pm 240$					
SR-2	$465 \pm 16$	$125 \pm 11$	$165 \pm 10$	$485 \pm 29$	$9,710 \pm 340$					
SR-3	$280 \pm 10$	2±6	$10 \pm 10$	$1 \pm 7$	$8,370 \pm 300$					
SR-4	$175 \pm 8$	$16 \pm 4$	4±7	$23 \pm 6$	$8,480 \pm 260$					
SR-5	$635 \pm 22$	$105 \pm 12$	$255 \pm 13$	$365 \pm 25$	$15,590 \pm 520$					
SR-5A	$620 \pm 23$	$185 \pm 16$	$170 \pm 14$	$310 \pm 24$	$10,840 \pm 420$					
G-1	$470 \pm 25$	17±5	$-2 \pm 5$	‡	$14,610 \pm 760$					
G-2	$620 \pm 35$	$83 \pm 13$	$51 \pm 6$	*	$12,090 \pm 660$					
Safe Harbo	or Dam									
SR-6	$765 \pm 26$	$20 \pm 9$	9±16	NAME OF TAXABLE PARTY.	$18,720 \pm 620$					
Holtwood 1	Dam									
SR-7	$395 \pm 15$	$67 \pm 10$	8 ± 12		$15,440 \pm 490$					
Peach Bott	om									
SR-8A	$795 \pm 17$	$15 \pm 5$	1 ± 8	*****	$18.260 \pm 510$					
SR-8B	$2,460 \pm 48$	$1,680 \pm 86$	$180 \pm 10$	28±9	$11,820 \pm 390$					
G-3	110±9	13±5	1±4		$8,230 \pm 450$					
G-4	$1,280 \pm 67$	870±94	$160 \pm 10$		$9,880 \pm 530$					
G-5	$210 \pm 14$	$115 \pm 14$	2±5		$6,260 \pm 360$					
G-6	$1,890 \pm 100$	$79 \pm 14$	5±9		$15,930 \pm 870$					
G-7	$535 \pm 31$	$415 \pm 46$	10±5		$7,790 \pm 440$					
G-8	$850 \pm 47$	$580 \pm 64$	40±8		$9,780 \pm 550$					
G-9	$315 \pm 17$	$180 \pm 20$	9±4		$5,780 \pm 300$ $5,840 \pm 300$					
Conowingo	Dam				ŕ					
SR-8	635 ± 22	$130 \pm 13$	12 ± 15	ND	$19.530 \pm 630$					
G-10	$535 \pm 32$	$220 \pm 26$	8±7		$16,230 \pm 880$					
G-11	$670 \pm 36$	$210 \pm 24$	6±6		$14,920 \pm 790$					
G-12	$1.070 \pm 58$	$490 \pm 55$	29±9	*****	$18,480 \pm 990$					
G-13	$710 \pm 44$	$160 \pm 24$	$-8 \pm 14$		$16,010 \pm 920$					
G-14	$960 \pm 51$	$450 \pm 48$	22±5	****	$16,420 \pm 860$					
Susquehani	na Flats									
SR-9	195±11	$110 \pm 10$	3±11		$11,600 \pm 370$					
G-15	$190 \pm 10$	145 ± 15	2±2	*****	$4,610 \pm 240$					
G-16	$390 \pm 22$	$280 \pm 32$	$-4 \pm 4$		$5.080 \pm 290$					
G-17	$135 \pm 10$	$105 \pm 13$	$-2\pm 7$		$13,520 \pm 710$					
G-18	$225 \pm 12$	$160 \pm 18$	2±3		$7.950 \pm 410$					
G-19	$330 \pm 17$	$265 \pm 21$	2±3 2±2		$7,930 \pm 410$ $3,940 \pm 210$					
G-19	$445 \pm 23$	$205 \pm 21$ $275 \pm 30$	5±2		$5,490 \pm 210$ $5,490 \pm 280$					
G-21	79±8	$\frac{273 \pm 30}{29 \pm 7}$	8±7		$11,770 \pm 630$					
G-22	790±43	$330 \pm 37$	8±8		$11,770 \pm 630$ $12,240 \pm 660$					
G-23	440±26	$140 \pm 17$	6±7		$9,290 \pm 510$					
G-23 G-24	76±5	$\frac{140 \pm 17}{27 \pm 3}$	3±2		$9,290 \pm 510$ $2,350 \pm 130$					
G-25	$480 \pm 26$	43±8	3±2 7±7	******						
G-25 G-26	480±26 480±27				$14,350 \pm 750$					
		100 ± 13	7±7		$11,940 \pm 640$					
G-27	$175 \pm 13$	$58 \pm 10$	$-4 \pm 8$	-	$14,260 \pm 760$					

Our radiocaesium profiles (Fig. 3) indicate that the areas behind Holtwood and Conowingo Dams are presently zones of rapid sediment and radionuclide accumulation. Gross et al. concludes that one-half to two-thirds of the sediment load of the lower Susquehanna is trapped behind the three hydroelectric dams and that  $\sim 1 \times 10^6$  Mg of suspended sediment are annually discharged past Conowingo Dam during years without major floods. The low activities for reactor radionuclides and the lack of radiocaesium in the core collected behind Safe Harbour Dam indicate that either this reservoir has not been a major trap for fine particles and radionuclides during the past two decades or our samples are not representative of the area as a whole. A detailed budget of sediment accumulation behind the dams based on radionuclide data from these and several more recent cores, is in preparation (J.F.D and O.P.B.)

The sediment and radionuclides which escape from behind Conowingo Dam seem to be efficiently trapped along the western margin of the Susquehanna Flats (Fig. 2) or in the turbidity maximum area (Core E) of Upper Chesapeake Bay. Goldberg et al.13 have observed sediment accumulation rates as great as 8 cm yr<sup>-1</sup> (and <sup>239,240</sup>Pu activity distributions to sediment depths as great as 70 cm) in the turbidity zone area near the location of Gravity Core E. The elevated PCB concentrations (0.1 p.p.m.) at 35-40 cm (ref. 15) in Core E and our <sup>137</sup>Cs profile support the conclusions of Goldberg et al. 13 that during the past two decades this area has been a zone of rapid sediment and contaminant accumulation.

The depth of <sup>137</sup>Cs penetration in Core 14, collected near Baltimore (Fig. 1), indicates that this may also be an area of recent particle accumulation. The lack of <sup>134</sup>Cs activity in the surface sediments of this core indicates that Susquehannaderived particles at this site are markedly diluted by sediments from other sources.

As most of the <sup>134</sup>Cs activity was only recently released from the PB reactor site, and because much of this <sup>134</sup>Cs activity seems to be confined to near the sediment surface (Fig. 3), we can estimate the total <sup>134</sup>Cs burden in the sediments by integrating the 134Cs activities in our grab samples and using these values as representative of different areas in the river and upper bay. A detailed budget of 134Cs accumulation, based on more samples than those presented here, is in preparation but preliminary estimates indicate that almost all the 134Cs released into the Susquehanna River has been trapped within the sediments upstream of Baltimore<sup>16</sup>

Our distribution data indicate that: (1) reactor-produced radiocobalt and radiocaesium are sequestered by particles and deposited in low-energy environments; (2) levels of contamination, though measurable, are a small fraction of the total radioactivity resulting from naturally occurring radionuclides; and (3) these nuclides can provide valuable insights into the transport and accumulation patterns of particles and particleassociated pollutants introduced into the Susquehanna-Upper Chesapeake Bay system.

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<sup>\*</sup> Activities decay corrected to date of sample collection, 22 May 1979 for SR samples and 26 September 1979 for G samples.

\* Natural \* Activities > 10,000 pCi kg<sup>-1</sup> indicate muddy samples.

Dashes indicate no 58 Co data, because the date of analysis was more than three half lives (273 days) after the date of collection.

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## Field-capacity water extracts from serpentine soils

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Soils derived from serpentinites and other ultramafic rocks have long been called 'serpentine soils' by biologists1. They often bear a distinctive vegetation with poor cover or rare species or both and have attracted botanical interest for centuries2.3. The chemical causes of the unusual vegetation have often been investigated using soil analyses of exchangeable and total quantities of ions or elements. These have shown that serpentine soils are rich in magnesium and have relatively high concentrations of nickel but low ones of calcium and other nutrients. Although these factors have been judged to be important in many serpentine soils, there have been few analyses of their soil solutions which would give a better assessment of likely soil toxicities and deficiencies. Moreover the soil solution can be effectively simulated in water-culture experiments to investigate directly the effect of each possible factor on plant growth. We report here (Table 1) the results of soil-solution analyses for a range of Scottish and Zimbabwean serpentine soils which bear unusual vegetation. These analyses have substantially altered previous conclusions concerning the chemical causes of serpentine vegetation7.

The soil solutions were extracted by centrifuging at 12,000g soil samples which had been maintained for up to 3 days at field capacity (moisture content after 24 h drainage). The solutions were analysed by standard atomic absorption techniques<sup>8</sup> for metals, the phenol disulphonic method8 (with modification in high chloride samples) for nitrate, the molybdenum blue method for phosphorus8 and by a silver titration method for chloride9. pH was measured using a glass electrode and bench pH meter.

The results are best discussed in the context of the three main chemical causes of plant growth on serpentines: high Mg/Ca quotient, high nickel and low nutrients.

The Meikle Kilrannoch soil is apparently uniquely toxic among British serpentines (J.P., unpublished results, and ref. 10) and there is evidence<sup>5,10-12</sup> to indicate that high magnesium is the prime cause of this toxicity. Magnesium toxicity depends on the concentration of calcium and, from soil exchangeablecation analyses it seemed that very low calcium exacerbates magnesium toxicity at Meikle Kilrannoch11. The soil-solution analyses (Table 1) offer a different explanation. The distinctive feature of the Meikle Kilrannoch soil solution is its high concentration of ions, particularly magnesium among the cations. Calcium is actually at higher concentrations than in other Scottish serpentines (in Table 1) but proportionally more calcium is required to ameliorate magnesium toxicity at higher solution concentrations of this element<sup>5,12,13</sup>

The soil-solution analyses have also been useful in judging the possibility that calcium deficiency may be an adverse factor in some serpentines. Several earlier workers had stressed the importance of calcium deficiency<sup>14,15</sup> but the demonstration of the very small absolute requirement for calcium by plants16 had suggested that calcium deficiency was unlikely to occur in serpentine soils. The lower soil-solution calcium concentrations at Coyles of Muick, Hill of Towanreef and particularly Green Hill (sites where soil exchangeable calcium is not very low<sup>17,18</sup>) still exceed the minimum calcium required by Zea mays 16. The possibility of calcium deficiency as a plant-determining factor in some serpentine soils should be reconsidered. Very low soil-solution calcium at Green Hill has been observed and three species of grass tolerant to this soil have root-surface

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Table 1 The means (±95% confidence limits) of ions (mg l<sup>-1</sup>), pH and Mg/Ca quotients in soil solutions in skeletal soils from serpentine sites in Scotland and Zimbabwe

Sites	No. of samples	Ni <sup>2+</sup>	Ca <sup>2+</sup>	$Mg^{2+}$	<b>K</b> ⁺	Na⁺	Cl <sup>-</sup>	NO <sub>3</sub>	PO <sub>4</sub> #	pH	Mg/Ca
Scotland	10	< 0.1	$0.80 \pm 0.21$	9.1 ± 1.2	$0.30 \pm 0.07$	45+074	$3.1 \pm 0.59$	52 ± 11	$1.3 \pm 1.0$	6.8±0.15	$13 \pm 3.7$
Coyles of Muick** Green Hill**	10 8	< 0.1	$0.80 \pm 0.21$ $0.45 \pm 0.12$	$9.1 \pm 1.2$ $9.4 \pm 1.8$	$0.36 \pm 0.13$			50 ± 12	$0.95 \pm 0.92$	$6.5 \pm 0.21$	$23 \pm 8.1$
Hill of Towanreef**	10	< 0.1	$0.80 \pm 0.31$	$12 \pm 3.9$	$0.58 \pm 0.29$		$8.3 \pm 9.5$	$57 \pm 7.6$	$3.2 \pm 1.7$	6.6±0.18	$18 \pm 7.1$ $5.0 \pm 0.66$
Keen of Hamar (Unst)*†‡	11	$0.13 \pm 0.04$	$5.4 \pm 2.3$	28 ± 15	$5.0 \pm 1.6$ $5.4 \pm 1.2$	$110 \pm 3.2$ $15 \pm 1.4$	200±72 ND	29±14 830±69	$0.97 \pm 0.10$ $13 \pm 5.0$	5.8±0.26 ND	3.0±0.00
Meikle Kilrannoch†§	11	$0.67 \pm 0.069$	$11 \pm 1.2$	$180 \pm 33$	5.4 ± 1.2	13 x 1.4	ND	030 ± 09	1525.0		
Zimbabwe Kingston Hill	4	$0.58 \pm 0.48$	$19 \pm 9.8$	$28 \pm 21$	$20 \pm 10$	ND	ND	ND	ND	ND	$1.5 \pm 0.36$
Tipperary Claims	3	$0.67 \pm 0.29$	$23 \pm 36$	$38 \pm 56$	$19 \pm 32$	ND	ND	ND	ND	ND	1.8±1.6

Samples collected moist in August 1980, stones > 1 cm diameter removed, wetted to field capacity a few days after collection and centrifuged after 1-3 days.

+ Chromium and cobalt analyses carried out on these samples: none exceeded the detection limit of 0.1 mg l

Samples collected from relatively dry soils in January 1977, sieved through a 2 mm mesh, air-dried, subsequent treatment as for Meikle Kilrannoch.

<sup>‡</sup> One sample was missing from the nitrate analyses. Three samples with nickel below the detection-limit (0.1 mg l<sup>-1</sup>) were not included in the calculation of mean and confidence limits for this element

<sup>§</sup> Samples collected moist in September 1976, sieved through a 2 mm mesh, air-dried, moistened to field capacity in laboratory in May 1977 and centrifuged after 3 days. ND, not determined.

hosphate species expressed as PO<sub>4</sub>

<sup>\*\*</sup> Nickel values were all below the detection limit (0.1 mg l<sup>-1</sup>) except for one sample from Green Hill with 0.18 mg l<sup>-1</sup> nickel.

phosphatases with activities unchanged in a range of calcium concentrations. Two grass species which grew poorly in Green Hill soil had calcium-sensitive root-surface acid phosphatases and it was inferred that the tolerant species were adapted to low soil calcium. In contrast, native Festuca rubra from Meikle Kilrannoch 19 has root-surface phosphatases with higher activities with increasing calcium, magnesium and nickel.

The use of water-culture experiments with Festuca rubra in media simulating soil solutions has shown that nickel is also likely to be toxic (at 0.7 mg l<sup>-1</sup> Ni<sup>2+</sup>), although less so than magnesium, at Meikle Kilrannoch<sup>13</sup>. Similar experiments with Agrostis stolonifera in simulated Keen of Hamar soil solutions have provided evidence of slight nickel toxicity (at 0.1- $0.2 \text{ mg l}^{-1} \text{ Ni}^{2+})^{20}$ . These experiments have allowed nickel toxicity in British serpentines to be assessed and helped to resolve conflicting evidence from soil and plant analyses and bioassays (J.P. unpublished data, and refs 10, 11, 18).

Earlier work in Zimbabwean serpentine soils had indicated the importance of nickel toxicity for plant growth<sup>21-23</sup>. Our soil-solution analyses support this conclusion, and show a low Mg/Ca quotient in the Zimbabwean samples, confirming that this factor is less important than a high nickel concentration.

The present results show that nutrient concentrations are not always low in serpentine soils. The nitrate concentrations at Meikle Kilrannoch are high and nitrate is the main balancing anion for magnesium at Coyles of Muick, Green Hill and Hill of Towanreef. Nitrate is lower at the Keen of Hamar. Here the site is close (all samples within 0.5 km) to the sea and the soil solutions resemble (with some enrichment of magnesium and nickel) ~1% seawater with Na<sup>+</sup> and Cl<sup>-</sup> as preponderant ions. Soil-solution phosphate concentrations in Table 1 are high but for this element the labile-pool size, for which we have no information, may be a more critical determinant of plant growth. Potassium concentrations range from low in the Scottish soils to moderately high in those from Zimbabwe.

Hydrogen and hydroxyl ions must contribute little to the total ion balance in these soil solutions as they are neutral to mildly acid. Table 1 shows direct measurements for four sites: measurements of soil pH (in a mix of equal proportions of soil and water) gave mean values of 6.7 for Meikle Kilrannoch, 6.1 for Kingston Hill and 6.2 for Tipperary Claims for samples collected close to those used for the soil-solution analyses.

The relevance of soil-solution extraction at field capacity can be questioned as soils are in this state for an unknown part of the growing season. Solution concentration can vary with the time of the year and with the method of extraction<sup>24,25</sup>. But cool moist conditions prevail at the Scottish sites and here the field-capacity extraction seems appropriate. Moreover, as the concentration of the soil solution increases on drying<sup>24</sup> the potentially toxic nickel in the field-capacity water extracts from the Zimbabwean samples represents a minimum level of nickel toxicity which must become much more acute. It is encouraging that F. rubra plants grown in water-culture media based on the field-capacity extracts from Meikle Kilrannoch soils have shown a broadly similar appearance, size and tissue element composition to the same species grown in the actual soils<sup>13</sup>.

Similar soil-solution analyses have been used successfully in recent studies of the vegetation of skeletal soils rich in copper, lead and zinc<sup>26,27</sup>. However, the interpretation of soil-solution analyses is less straightforward for soils with much organic matter where heavy metals are likely to be in complexes of unknown chemistry and toxicity19

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## **Upper Cretaceous to Eocene pelagic** limestones of the Scaglia Rossa are not Miocene turbidites

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The Scaglia Rossa is a pink limestone composed of 1-20% forams and  $\sim$ 5% clay in a coccolith matrix that outcrops extensively in the Umbria-Marches Apennines of northern peninsular Italy. It has universally been considered to be a pelagic sedimentary rock deposited during the late Cretaceous to middle Eocene. The Scaglia Rossa has been studied intensively as a record of micropalaeontological<sup>1-7</sup>, palaeomagnetic<sup>8-36</sup>, sedimentological<sup>31-34</sup>, and geochemical<sup>33,35-39</sup> events. Recently, Wezel<sup>40</sup> reported that the limestones are not of pelagic origin but are entirely turbiditic, and were deposited in the Miocene. He also criticized magnetic polarity stratigraphy investigations done on the Scaglia Rossa, concluding that the polarity is a function of sedimentation rate, not of geomagnetic field polarity. His evidence has cast doubt on previous stratigraphic information from the Scaglia Rossa<sup>41</sup> and compels us to respond. Although the observation by Coccioni<sup>42</sup> and Wezel<sup>40</sup> of the presence of fossils of anomalously young ages in clay seams between the limestone beds is of interest, we show here that the proposed redating of the Scaglia is wrong for several palaeontological, sedimentological and palaeomagnetic reasons.

Wezel stressed the importance of Coccioni's 42 first report of Miocene planktonic forams in clay partings between limestone beds in the Scaglia<sup>40</sup> but did not comment on Premoli Silva and Luterbacher's<sup>43</sup> conclusion that these volumes forems contaminants.

On checking these observations we have found that Miocene forams are indeed present in clay interbeds in various places. However, detailed examination of ~200 thin sections of Scaglia limestone beds from six localities, including 57 thin sections from the Bottaccione Gorge at Gubbio, spanning the same stratigraphic interval as Coccioni's 42 clay samples, did not reveal a single Miocene foram, although the spherical forms Orbulina and Praeorbulina, the most common types identified by Coccioni, yield distinctive circular sections. Wezel<sup>40</sup> found no detrital grains accompanying the younger forams, and because these forams are the same age as the turbiditic Marnosoarenacea Formation, which is rich in quartz and mica grains, he concluded that the Miocene forams could not be contaminants derived from the Marnoso-arenacea. However, in a test of this conclusion carried out at the University of California, A. Montanari (personal communication, 1981) washed clay from the Cretaceous-Tertiary boundary in the Bottaccione section at Gubbio and found rare quartz grains and particles of cemented quartz sandstone and siltstone, some of the latter actually containing Miocene planktonic forams cemented into the siltstone. He also found these materials by washing soil collected on the Scaglia Rossa outcrop face. These observations are striking proof that the Miocene forams are contaminants derived from the Marnoso-arenacea.

Wezel<sup>40</sup> illustrates one thin section from a hard limestone bed of the basal Palaeocene Globigerina eugubina zone showing a foram which he first identifies as the Upper Palaeocene to Middle Eocene planktonic form "Globorotalia ex gr. G. aragonensis - G. formosa" and later as "Globorotalia cf. aragonensis"; "ex gr." (out of group of) and "cf." (compare) indicate that the fossil could not be precisely identified. However, according to Premoli Silva (personal communication, 1980), the wall structure of this foram indicates that it is a benthic, not a planktonic type; it probably belongs to the genus Congotalites.

The Scaglia Variegata, Scaglia Cinerea and Bisciaro units, which overlie the Scaglia Rossa, have been found to contain an orderly sequence of Upper Eocene–Lower Miocene forams and coccoliths<sup>44–47</sup>. In Wezel's interpretation all previous work on these units must be wrong.

Wezel<sup>40</sup> considers that the Scaglia Rossa is a Cretaceous-Palaeocene sediment redeposited by turbidity currents during the Miocene. He therefore stresses the occurrence of turbiditic beds in some sections of the Scaglia Rossa. These beds, showing grading, sole marks and Bouma sequences, and described as 'detrital beds', 'calcarenites', or 'turbidites', are usually conspicuously white in a formation that otherwise is dominantly pink. Wezel<sup>40</sup> goes beyond other workers in considering other parts of the Scaglia Rossa to be of turbiditic origin. Describing the Bottaccione section at Gubbio, which all others consider to be entirely pelagic, Wezel<sup>40</sup> says: "Except for some lamination, calcareous beds do not seem to exhibit obvious turbidite structures. Bedding-parallel stylotites and recrystallization obli-terated the possible structures." Nevertheless, he infers a "turbidite distal setting for the calcilutites of Bottaccione". He suggests that a 350-m sequence of foram-coccolith limestones, showing a normal 50 Myr sequence of foraminiferal biozones<sup>4</sup> is composed entirely of turbidites, when there has been no report of any turbiditic feature. A more probable conclusion is that the Scaglia Rossa is a pelagic sediment in which there are local intercalations of calcareous turbidites.

All palaeontologists who have studied the Scaglia have recognized an orderly sequence of Upper Cretaceous to Middle Eocene foraminiferal zones<sup>1-7</sup>. Even Wezel<sup>40</sup> admits that the Scaglia shows a normal "thin section biostratigraphy". If the Scaglia is Miocene, the orderly Cretaceous to Eocene foraminiferal zonation must be explained. Wezel<sup>40</sup> explains the gross normal 'thin section biostratigraphy' with the intervention of an intermediate area of sedimentary accumulation, located between the source area and the final basin of Scaglia Rossa deposition. This means that after deposition of a normal stratigraphic sequence of Cretaceous-Eocene pelagic carbonate ooze, successive layers were stripped off and redeposited by turbidity currents in an intermediate basin. This would invert the order of biozones. Subsequently the process was repeated, so that in the final deposit—the present-day Scaglia—the original order of biostratigraphic zones was restored (Wezel, personal communication, 1979).

Although Wezel<sup>40</sup> states that this is not an extravagant hypothesis, mobilization of turbidity currents simply does not occur by the stripping off of broad but very thin sheets; it is a process which inevitably mixes material from a considerable stratigraphic thickness of the source body. Yet Wezel requires that this happen twice with no increase in the disorder of the sedimentary sequence.

Five sections of Scaglia Rossa, including the Bottaccione section, have been found to contain an abnormal abundance of iridium precisely at the Cretaceous-Tertiary boundary 35,36,39. Similar iridium anomalies occur at the Cretaceous-Tertiary boundary in several other locations (Denmark, at the type

Danian locality<sup>35,36,39,53,64</sup>, southern Spain<sup>54</sup>, northern Spain<sup>55</sup>, New Zealand<sup>39,55</sup>, and the north-central Pacific<sup>64</sup>). As iridium is greatly depleted in the Earth's crust relative to its cosmic abundance, the anomaly has been taken to indicate an influx of extraterrestrial material at the time of terminal Cretaceous extinctions<sup>35,36,39</sup>. If the Scaglia Rossa is really Miocene, it must be coincidence that an iridium anomaly occurs just where the reworked Cretaceous and Palaeocene forams suggest that we are seeing the Cretaceous–Tertiary boundary.

Wezel dismisses the concordant palaeomagnetic results of four independent groups who, since 1973, have measured several thousand samples, and agree that the Scaglia Rossa is an excellent recorder of the magnetic field<sup>8-30</sup>. Umbrian palaeomagnetic directions from Jurassic to Palaeocene define a segment of an apparent polar wander path which, after correction for a tectonic rotation reflecting the overall geodynamic history of the region, is consistent with the reconstructed African polar wander path for that time interval<sup>28</sup>.

The Scaglia Rossa has yielded a magnetic polarity record that exactly matches the seafloor spreading magnetic anomalies in the parts of the ocean known from deep-sea drilling to be of late Cretaceous and early Tertiary age 16,23. Subsequently we have found in the Umbrian sequence an essentially perfect match from anomaly M-4 (Barremian) to anomaly 6C (basal Miocene), an interval of about 100 Myr (refs 29, 30, 46, 47, 63, 65). Furthermore ~80% of this reversal sequence has been checked by measuring more than one section. The same magnetic reversal sequence is found in the Southern Alps, exactly correlated with the biostratigraphic sequence from studies in the Hauterivian through the Maastrichtian Magnetic stratigraphic studies on DSDP Legs 73 and 74 have further confirmed parts of the reversal sequence overwhelming evidence that the Scaglia Rossa is not Miocene.

Wezel's explanation of this polarity stratigraphy is that normal directions occur in thin beds whereas reversed directions occur in thick beds. His Fig. 4 shows columns giving magnetic properties and bedding thickness at Gubbio and he states that they correlate, but we fail to detect any correlation in this figure. The intensity of magnetization of a sediment depends on the concentration of magnetic minerals, which might be related to bedding thickness. However, the direction of remanence is acquired by post-depositional alignment of magnetic grains in water-logged interstices below the sediment-water interface and is independent of the magnetic mineral concentration 10,58-61. Therefore, even if a correlation between remanent intensity and bedding thickness were to be demonstrated, this would be unrelated to the polarity sequence.

If the Scaglia Rossa is Miocene, and if the Scaglia Bianca is correctly dated as Cenomanian (F. C. Wezel, personal communication, 1979), there must be a hiatus of ~80 Myr between the two formations. Wezel indicates that the hiatus is hard to find because the Scaglia Bianca—Scaglia Rossa sequence is paraconformable. We note that the pattern of magnetic declinations and inclinations shows no break in the vicinity of the Bianca—Rossa contact<sup>24,26</sup>, which would be extremely improbable if there were a hiatus of 80 Myr hidden in the sequence. Moreover, 100 m of normally polarized limestone lie above the Scaglia Bianca—Scaglia Rossa contact<sup>21,23,24,26,29</sup>. This corresponds to the later part of the Cretaceous magnetic quiet zone. There is no equivalent to this long normal zone in the Miocene reversal history<sup>62</sup>.

If the Scaglia Rossa is Miocene, the perfect match between the Umbrian magnetic stratigraphy and the sea-floor anomalies for almost 100 Myr must be another coincidence unless we consider that all of the sea floor currently considered to be of late Cretaceous to Oligocene age is actually of Miocene age. Many DSDP Initial Reports document that this is not the case.

It has been suggested that the Miocene forams between limestone beds at Gubbio might indicate that portions of the Scaglia Rossa are huge osteoliths. The palaeomagnetic stratigraphy shows that this is not the case. Declinations change

slowly and smoothly; they do not show the sudden breaks that should occur at the limits of olistostrome blocks, at least some of which would have rotated during emplacement. Rotations of this kind have been found in several synsedimentary slide masses 5-15 m thick in the turbidite-bearing Furlo section<sup>63</sup>; they are not present at Gubbio, or in any of the entirely pelagic sections which have been studied.

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### A reply by F. C. Wezel

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I thank Alvarez and Lowrie for their comments on my paper on the Scaglia Rossa<sup>1</sup>. However, I found their comments scientifically unsatisfactory for at least two reasons. First, although my work was published two years ago they present no new observed facts. Rather they seem to have adopted a model to which all data must be fitted. Second, although I raised the problem of Miocene foraminifera in the Scaglia Rossa turbidites, I did not, in fact, conclude that they were Miocene in age.

Further, Alvarez and Lowrie seem to have misinterpreted the spirit of my paper. In dispelling the alleged cliché of the Scaglia Rossa as "a conformable, complete and pelagic sequence of oceanic type"2,3, I stressed the rudimentary state of our factual geological knowledge of the formation and the urgent need for

more research "before continuing to pursue other striking speculations remote from facts". It is regrettable that Alvarez and Lowrie have ignored some basic points of my paper contradicting the inferred pelagic origin of the Scaglia Rossa limestones. For example, (1) The palaeogeographic reconstruction indicating a series of narrow and current-influenced troughs in a probable taphrogenic regime, marked by a combination of subsidence and distension. (2) The facies and palaeocurrent analysis showing the distal basinal environment of the Bottaccione section. (3) The basin-wide regional megarhythmicity of the vertical sequences, expressing temporal changes in sedimentation rate as a consequence of the structural activity of the passive margin tectonics.

The micropalaeontological data of Coccioni (personal communication) clearly show the presence of Miocene foraminifera in clay interbeds from various sections of the Scaglia Rossa. This work was carried out in our laboratory by painstakingly washing 400 kg of sample; 238 Miocene foraminiferal tests were recovered. Alvarez and Lowrie's statement that "not a single Miocene foram was found . . . in detailed examination of about 200 thin sections of Scaglia limestone beds from six localities ... ", is not at all surprising. It rather pinpoints the methodological inadequacy of thin section studies. The presence of Miocene microfossils represents undoubtedly a real problem which has not yet been solved. With our current knowledge, I am now inclined to believe that the Scaglia Rossa is probably Cretaceous-Eocene in age, but complete disregard of the observed younger foraminifera is not an adequate solution to the problem.

Alvarez and Lowrie did not take into account a vast fund of geological information. They state once more the presence of an anomalously high iridium concentration at the Cretaceous-Tertiary boundary in the Bottaccione and other four sections of Scaglia Rossa. They stated that "No iridium anomaly is yet known at any other level in any pelagic sediment". Possibly one could believe in such "iridium anomaly" after many analyses have been undertaken in other beds of the sedimentary column (see ref. 4). Unfortunately, however, the concerted effort of the catastrophists<sup>5-7</sup> focused almost exclusively on the 'magic' thin Cretaceous-Tertiary boundary clay. In such conditions, without a thorough and detailed examination of numerous other clay interbeds and consideration of the minero-petrographic and geochemical contexts, it would be wrong to interpret a unique event as a catastrophe.

Some very significant recent results have now demonstrated other iridium anomalies at different levels. For example, a series of analyses carried out on the 1-m thick cherty black shale ('Bonarelli level', considered Turonian in age), located ~ 240 m under the Cretaceous-Tertiary boundary, has unequivocally shown an anomalously high iridium concentration, about twice as great as the K/T clay8. Recent geochemical and petrographic investigations9 suggest that this iridium anomaly could be imputed to volcanic activity and not to a second even more drastic extraterrestrial catastrophe.

Finally, I should like to re-emphasize the crude relationship I observed between magnetic properties and variations in lithology. Palaeomagnetic data from the thick coarser-grained turbidites may be controlled by depositional currents, whereas those from the finer-grained beds may more accurately record the Earth's magnetic field.

Therefore the conclusions by Alvarez and Lowrie do not seem to be supported by adequate facts and thus the 65-Myr catastrophic event may be considered an imaginative but unproven hypothesis. I therefore urge, once again, a critical assessment of the different aspects of the Scaglia Rossa geology.

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## Population trends among Jamaican reef corals

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Disturbance has been cited as a potentially important agent in structuring ecological communities by modifying the effects of competition1-5. Catastrophic disturbance has also been proposed as a factor promoting the coexistence of competing species in highly diverse tropical ecosystems such as rain forests and coral reefs<sup>2,6-11</sup>. Here we describe patterns of recruitment and mortality among reef corals over 4 yr at several depths on the reefs of Discovery Bay, Jamaica, which were struck by Hurricane Allen on 6 August 1980<sup>12</sup>. Photographic quadrats monitored since 1976 on a shallow water reef showed a negative correlation between coral abundance and mortality which was not offset by compensatory patterns of recruitment. This slow trend in the disproportionate reduction of rarer, competitively inferior species was reversed by Hurricane Allen, with storminduced mortality being greatest in the most abundant species. On deeper reef stations, undisturbed by the storm, slower rates of colony loss were compensated for by commensurate rates of colony recruitment. Thus, patterns of differential mortality and recruitment contribute to the maintenance of high species diversity in this tropical marine ecosystem.

Photographic stations were established on Monitor Reef, on the West Fore Reef of Discovery Bay, a well studied area<sup>13</sup> of classic Caribbean reef morphology<sup>14,15</sup>, in September 1976, and were rephotographed biennially until 1980 (Fig. 1). Photographs were analysed for per cent cover, species number and relative abundance within the plots, recording the fates of 1,718 individual coral colonies in 24 species with respect to recruitment, whole and partial mortality, and growth.

Reef-building corals are photosynthetic and compete to secure space for the interception of both light and plankton 16-19. Competitive outcomes between neighbouring individuals are determined by differential growth rates, overtopping morphologies and abilities in extra-coelenteric digestion<sup>2</sup> Before Hurricane Allen, mortality among shallow water corals from photostations II (3.8 m depth) and IV (6.7 m depth) was inversely density dependent, with species of lesser abundance suffering proportionally greater percentages of loss due to partial as well as complete mortality of individual colonies (Fig. 2). Recruitment rates were low for all species in shallow water stations; recruitment rates to photostations II, IV and XII of newly arriving colonies which achieved a size of ≥ 1 cm<sup>2</sup> ranged from 0.0 to 1.81 m<sup>-2</sup> yr<sup>-1</sup>, with an average rate for all 24 species in the 4-yr survey before Hurricane Allen of  $0.19\pm0.39~\text{m}^{-2}~\text{yr}^{-1}$ . In addition, the disproportionate rate of loss among the rarer species was not offset by compensatory recruitment patterns. These data suggest that in the absence of a major perturbation, there is a tendency for shallow-water communities to become monopolized by the branching Acropora species, causing a reduction of diversity with time.

Hurricane Allen was the second strongest recorded in the New World this century<sup>12,23</sup>. It was designated as a 5 on the International Hurricane Scale (there is no 6), with central pressures of 899 mbar and maximum wind speeds of 285 km h<sup>-1</sup> (ref. 24). The north coast of Jamaica, normally sheltered from severe storms<sup>25</sup>, received storm waves ranging from 6 m height over the phototransect area to 12 m height on less sheltered

reefs on the eastern side of the bay. (The last hurricane with similar track to Allen was in 1917<sup>26</sup>. However, disturbance sufficient to set back *Acropora* dominance may occur in severe winter storms at greater frequency.) Damage to corals varied with their depth of attachment, exposure to impinging waves, skeletal tensile strength and morphology<sup>23</sup>. Analysis of the storm effects in the phototransect areas reveals that in water less than 10 m deep, mechanical disturbance from fracture, scour and toppling reduced living coral cover from 51–54% to 10–12%. Mortality was greatest among the branching, friable and loosely attached *Acropora* species<sup>27–29</sup> which numerically and areally dominated the pre-hurricane reef. During the first 4 yr of the survey, we documented an increase in dominance by these species, a trend which was reversed during Hurricane Allen (Fig. 2). This reversal in mortality patterns does not result from

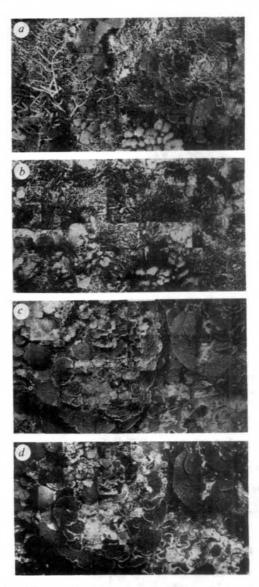


Fig. 1 a, Photostation IV at 6.7 m depth, Monitor Reef, Discovery Bay, Jamaica, 28 September 1976; b, same station, 14 August 1980, 8 days after Hurricane Allen. c, Photostation XII in 32.3 m depth, Monitor Reef, 1 October 1976; d, same station, 14 August 1980, post-Hurricane Allen. Photostation montages shown are constructed from 32 individual 0.5 × 0.5 m photographic frames that cover a 2 × 4 m plot; 8 m² is sufficient to define per cent cover, relative abundance, cumulative species number and average number of colonies per m² such that a doubling of this sample size would not be expected to change the value of the measured parameter by more than 5% (ref. 34). In comparing a and b, note the differential removal of the spatially dominant branching Acropora species and the creation of a pavement of fragmented coral branches.

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Acropora commonness per se but from its morphology and concomitant susceptibility to wave damage.

In the deeper reef station (PS XII, 32.2 m depth), our detailed analysis did not reveal any effects of the storm (Fig. 1c, d). In the 4 yr before Hurricane Allen, no apparent relationship existed between the per cent cover of a coral species and the likelihood that it would loose or gain area in a subsequent survey; nor did this pattern change in the immediate post-hurricane survey. Recruitment patterns have not varied from year to year in the deep reef station, but, in contrast to recruitment patterns in shallow water which showed no relationship between loss and recruitment, there is a highly significant (Spearman rank correlation  $r_s = +0.55$ ; 0.01 > P > 0.005) positive correlation between the loss of colonies and their recruitment into the deeper reef station.

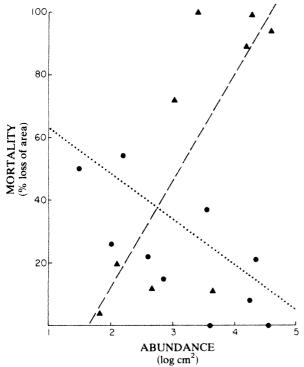


Fig. 2 Loss of existing area by each species in a subsequent survey ( September 1976 to September 1978, pre-Hurricane Allen; A. September 1978 to August 1980, post-Hurricane Allen) is plotted against the abundance of that species in shallow-water photostations II and IV. Photographs are analysed from area covered by each separate colony with a Hewlett-Packard 9815A-9864A digitizer. Mortality was measured as the disappearance of colony area from one survey to the next and includes mortality from physical factors, disease, predation, extra-coelenteric digestion and occlusion from the camera's eye due to the overgrowth of one species by another. We assume overtopping reduced fitness of fully shaded lower individuals and have demonstrated in photostations II and IV that the removal of canopy branches of Acropora palmata reveals many dead coral colonies which were living just before being overtopped between 1976 and 1980<sup>23</sup>. Mortality was negatively correlated with abundance for the years preceding Hurricane Allen (Spearman rank correlation  $r_s = -0.74$ ; 0.01 > P > 0.005; n = 10). While all species lost some area during the storm, the pre-hurricane pattern of disproportionate loss among the rarer coral species was reversed, with natural selection significantly favouring corals of lesser abundance ( $r_s = +0.65$ ; 0.05 > P >0.025; n = 9). Trend lines are determined by linear regression. Rank order for adequately sampled coral species (three or more colonies and at least  $1.0\times10^2~\text{cm}^2$ ) for photostations II and IV for both years is: A. palmata (PS II), Acropora prolifera (PS IV), A. palmata (PS IV), Montastrea annularis (PS IV), Acropora cervicornis (PS IV), Agaricia agaricites (PS IV), A. agaricites (PS II), Porites astreoides (PS II), M. annularis (PS II) and Millepora complanata (PS IV). The rarest species included in the initial survey (1976-78), M. complanata, was too rare to sample adequately in the subsequent survey (1978-80) because it was almost completely overgrown by 1978.

Hurricane Allen has produced a complex biological and morphological rearrangement on the Jamaican reef, in striking contrast to the original organization. Before the storm, shallow water mortality was strongly influenced by biological controls of competition through overgrowth; during the storm, mortality was predominantly controlled by physical factors, with heaviest losses among the commonest, competitively superior (but with respect to the storm, morphologically inferior) species. Hurricane Allen has temporarily eliminated overtopping as a significant agent of mortality in shallow water Jamaican reefs. Although patterns of mortality reversed during the storm, it must be kept in mind that the post-hurricane shallow reef retains slightly less than 10% of the orginal coral cover, and the predictive model (exemplified by Fig. 2) that diversity will increase until crowding slowly reduces it, remains untested by recovery data. Despite the proven regenerative powers of coral fragments<sup>29-32</sup>, in the period following the hurricane there has been a high mortality among Acropora branchlets tagged or photographed immediately after the storm<sup>33</sup>. It remains to be demonstrated whether any of these asexually produced pieces will survive to contribute significantly to the regenerating reef.

In deeper water, more equitable competitive abilities among species and slower growth rates could retard competitive exclusion, allowing lower disturbance levels to act successfully as diversifying forces<sup>2,4,7</sup>. Compensatory processes of recruitment, quantified in the present study, might also promote species richness.

This report is part of a long-term study of changes in community structure. It contrasts those changes occurring under the slow influence of continuously acting or chronic factors with those due to sudden but rare catastrophic disturbances. We are continuing to document the successional dynamics of recovery after this natural experiment so as to quantify better the mechanisms by which patterns in these communities arise, persist or change.

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## Evidence for delayed mortality in hurricane-damaged Jamaican staghorn corals

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Severe tropical storms can cause widespread mortality in reef corals<sup>1,2</sup>. The Caribbean staghorn coral, Acropora cervicornis, although dependent on fragmentation for asexual propaga-, is particularly vulnerable to hurricane damage<sup>6,7</sup> most important agents of post-hurricane mortality are assumed to be high wave energy and change in salinity, factors which typically soon diminish in intensity. We report here that there was substantial delayed tissue and colony death in A. cervicornis on a Jamaican reef damaged by Hurricane Allen. This previously undocumented degree of secondary mortality, sustained for 5 months and unrelated to emersion9, was over one order of magnitude more severe than that caused by the immediate effects of the storm. The elimination of >98% of the original survivors suggests potentially complex responses to catastrophes, involving disease 10,11 and predation, which may explain the widely variable rates of reef recovery previously reported 12-15.

Hurricane Allen passed within 65 km of the reefs of Discovery Bay on 6 August 1980 (refs 7, 16). This storm, the strongest recorded for the Caribbean, generated massive waves (>6-12 m) but little rain<sup>7</sup>. The large mounds of the delicately branching A. cervicornis, which had dominated the West Fore Reef between 6 and 20 m depth<sup>4,17</sup>, were extensively fragmented<sup>7,16</sup>. To assess the long-term effects, we surveyed all pieces of A. cervicornis having living tissue in three haphazardly chosen 1-m<sup>2</sup> areas of moderate to severe damage at 8 (n = 125)and 14 (n = 129) m depth on Dancing Lady Reef 3-9 days after the storm. These pieces were all detached by the hurricane, the skeletal break often (53%) occurring where there had been no living tissue<sup>4</sup>. Although the once open network of branches was compacted, much of the surviving tissue was not smothered by direct contact with other fragments or the underlying solid reef base. The median number of living or partially living branches per fragment was 3 (range 1-19), and 53% of the fragments had at least one living axial (terminal) polyp (median = 1, range 0-17). The median total length of living tissue in these quadrats was 916 (range 457–1,250) cm  $m^{-2}$ , ~40% of 1977 estimates<sup>1</sup> We individually tagged each living staghorn in the six quadrats and returned at irregular intervals to re-examine them.

Mortality continued at an unexpectedly high rate (Fig. 1). For censuses made within 4 weeks of the storm, there were highly significant differences at both depths (after tagging) between surviving and dead colonies in the lengths (when tagged) of their longest living branches (P < 0.001, Mann-Whitney U test), but this difference showed reduced significance by 7 weeks (P < 0.03) and was absent by 12 weeks (P > 0.1). The relationship between the total number and presence of axial polyps on the longest living branch and survival was not strong (0.05 > P > 0.01 at any depth for one census only). Other attributes (depth, branching order<sup>19</sup>, number of live branches, orientation of longest living branch and relative height of fragment off bottom) showed no significant relationship with survival. Five months after the storm, we found only four living colonies within the six

quadrats, and median total length of living tissue was reduced, from 916 to 10 (range 0-26) cmm<sup>-2</sup>. Thus there had been a nearly 100-fold long-term decrease in living colonies from the original post-storm number. Examination of other areas on Dancing Lady and other Discovery Bay reefs indicated that our quadrats were not atypical.

Two 18-m<sup>2</sup> transects were established near the shallow and deep sites following the death of most of the originally tagged corals by January 1981. Surveys of these transects showed that, in some areas, tissue mortality still exceeded new growth (indicated by lengthening branches and production of new axial polyps) nearly 1 yr after the hurricane. At 8 m depth there was a 26% reduction in live tissue (from 27 to 20 cm m<sup>-2</sup>) between mid-March and late July, while at 14 m depth there was an increase of 11% (from 26 to 29 cm m<sup>-2</sup>). In both transects the number of living fragments had declined during this interval (from 25 to 17 at 8 m, and from 23 to 17 at 14 m).

There are many possible explanations for this delayed mortality. Fragmentation might (1) facilitate continuing abrasion by the rolling of free fragments<sup>12</sup>; (2) decrease the 'three-dimensionality' of the reef, resulting in smothering or easier access for benthic predators of corals<sup>4</sup>; or (3) increase the susceptibility or exposure of colonies to disease 10,11. A. cervicomis could be particularly susceptible to these processes because of its growth form, sensitivity to stress (for example, extreme temperature 20,21 and low salinity8), and its apparently high vulnerability to disease 11 (perhaps partly a consequence of its tendency to form large stands, hence increasing the potential for epidemics<sup>22</sup>). Differential initial mortality of staghorn coral and its predators, competitors<sup>23</sup>, prey and pathogens could upset pre-storm relationships, leading to the coral's sharp decline. Some of these mechanisms seem to have had no major effect: we observed little evidence of burial by sediment or continuing mechanical abrasion, even on days with large swells. There was no obvious, long-term increase in competition with algae; species which showed 'blooms' within a month of the hurricane have since died back.

During the first few months, much of the mortality was probably related to continuing disease and fragmentation stress. Unusual amounts of tissue exfoliation, resembling that termed the white band disease' were observed in some colonies of A. cervicornis for 2 months before the hurricane. This exfoliation continued after the storm, and could not be reliably distinguished from predation (see below) for at least 1 month. Although a relationship between fragmentation and disease has been reported for A. cervicornis in Curacao. We found no persistent relationship between survival and fragment length, orientation or elevation. There is, moreover, some evidence for delayed post-hurricane mortality in species of Acropora not showing extensive tissue loss beforehand (Acropora palmata in Jamaica and Acropora prolifera in St Croix; R. D. Clarke, personal communication).

By January 1981 mortality was primarily related to the activities of species that were previously important sources of

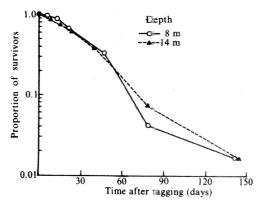


Fig. 1 Proportion of fragments surviving as a function of the time elapsed after tagging. Each fragment had one or more patches of living tissue, and was tagged 3-9 days after the hurricane.

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Table 1 Estimates of mean predator densities (per m<sup>2</sup>) before and after Hurricane Allen

	Density (m <sup>-2</sup> )	
Depth,	(Before	(After
Location	hurricane)	hurricane)
8 m, DL	13.5 (30)	8.4 (16)
14 m, DL	6.0(30)	2.6 (25)
14 m, DL	1.8(30)	1.3(25)
9 m, LTS	0.6(40)	0.3(25)
14 m, DL	0.6 (40)	0.1 (200)
	Location 8 m, DL 14 m, DL 14 m, DL 9 m, LTS	Depth, (Before hurricane)  8 m, DL 13.5 (30)  14 m, DL 6.0 (30)  14 m, DL 1.8 (30)  9 m, LTS 0.6 (40)

Reefs surveyed were Dancing Lady (DL) and LTS (reef immediately west of DL); numbers in parentheses indicate numbers of 1-m<sup>2</sup> areas examined. The only values available for before the hurricane are based on surveys by Kaufman<sup>24</sup> (1977; *Diadema, Eupomacentrus*) and L. S. Land (unpublished results, 1975; Coralliophila), but these seemed to be representative of pre-storm conditions in 1980. 'After' densities were assessed in February-March 1981.

mortality in Discovery Bay. Predation by the snail Coralliophila abbreviata 4,24,27 has been particularly severe in shallow water; 1-m<sup>2</sup> quadrats along the transect at 8-m depth containing living staghorn and snails in May showed a median decrease by July in total staghorn length of 27.5 cm, whereas quadrats containing coral but no C. abbreviata showed a median increase of 2 cm (P < 0.01, Mann-Whitney U test). Disturbance by the urchin, Diadema antillarum<sup>24,25</sup>, and the damselfish, Eupomacentrus planifrons<sup>24</sup>, has also contributed to the observed coral deaths. In March, densities of these species were generally lower but within an order of magnitude of pre-storm estimates (Table 1), in contrast to the 100-fold decrease suffered by the staghorn. Thus predation pressure per colony of surviving A. cervicornis is now substantially higher.

Several important points emerge from this study. First, future studies of catastrophic damage to reefs (and other ecosystems) should be started as soon as possible after the event. By 5 months, for example, many tagged dead pieces had eroded calices, thus this characteristic<sup>14</sup> could not by then be used to distinguish between pieces dying before and after the storm<sup>2</sup> Second, the geographical and temporal scale over which catastrophes occur could critically influence subsequent events. For example, destabilization of relationships between corals and their predators is more likely to occur when the area of coral destruction is great; adult predators of limited mobility, unable to emigrate from decimated regions, will continue to feed in areas where new coral growth can no longer keep pace with predation. The resulting drop in coral abundance will be particularly severe if predators do not succumb rapidly to starvation. Similarly, when substantial coral mortality (for whatever reason) occurs only rarely, the relative abundances of associated reef organisms are more likely to be strongly shifted28. Thus survival probabilities and regeneration rates observed after routine levels of fragmentation (experimental or natural<sup>4,5,11</sup>) may tell us little of the reef's potential for recovery from unusually exten-

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## Carbon-13 isotopic fractionation as a measure of aquatic metabolism

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The fixation of dissolved CO<sub>2</sub> in organic matter during photosynthesis preferentially removes 12C from the water, causing the remaining HCO<sub>3</sub> to become enriched in the less abundant isotope, <sup>13</sup>C. Respiration later releases part of this CO<sub>2</sub> back into the water. Metabolically active aquatic communities thus can generate variations in both the chemical1-3 and isotopic compositions of the water surrounding them. We describe here experiments designed to establish the isotopic fractionation of carbon by the metabolism of two common coral reef organisms. We then show that the fractionation coefficient obtained for the metabolism of those organisms is also applicable to a reef community and may be used to estimate the metabolic rate of the community.

In our first experiment, we placed specimens of the common Indo-Pacific reef-building coral, Pocillopora damicornis, in a 90-litre aquarium and alternately placed the aquarium in sunlight and darkness during a 6-h period. The aquarium was covered with a sheet of clear plastic (Mylar) to impede gas exchange across the air-water interface. Samples were collected for measurement of pH and alkalinity and calculation of ΣCO<sub>2</sub> (ref. 8), and duplicate samples were processed for carbon and oxygen isotopic analyses9. We shall consider here only the carbon data. The isotopic data are reported in the familiar  $\delta$  notation:  $\delta^{13}C = [(R_{\text{samp}} - R_{\text{std}})/R_{\text{std}}] \times 1,000$  where R is the ratio  $^{13}C/^{12}C$  and  $\delta^{13}C$  is reported in parts per thousand (%) relative to PDB. The second experiment was similar, except that we used the common reef-flat red alga, Acanthophora specifera, and the incubations occurred over a 24-h period.

In the third experiment, water samples were collected from a coral reef in Kaneohe Bay, Hawaii, over a mixed algal/sand community in water about 3 m deep. Procedures were similar to those used for the two aquarium experiments, except that the water over the reef community was open to gas exchange with the atmosphere. The observed range of  $\Sigma CO_2$  variation in the field experiment was about 80 µmol l<sup>-1</sup> compared with 300 µmol 1<sup>-1</sup> in the aquarium experiments. Water flow across the reef is typically < 5 cm s<sup>-1</sup>. Therefore samples were collected at one location at selected 1-h intervals over a 24-h period.

Consider the simplified metabolic reaction:

$$CO_2 + H_2O = CH_2O + O_2$$
 (1)

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The progressive utilization of  $\Sigma CO_2$  as the reaction proceeds from left to right represents photosynthesis and should be described by a change of  $\delta^{13}$ C as a function of decreasing  $\Sigma$ CO<sub>2</sub>. Similarly, respiration (the metabolic reaction proceeding from right to left) should be described by a change of  $\delta^{13}\bar{C}$  as a function of increasing  $\Sigma CO_2$ .

Broecker and Oversby 10 demonstrate the use of the Rayleigh distillation equation to describe the progressive change in the isotopic composition of some material, X, as that material is removed from the initial dissolved phase,  $X_0$  according to a single-stage fractionation model:

$$\frac{R_X}{R_{X_0}} = f^{\alpha - 1} \tag{2}$$

where  $R_{x_0}$  is the starting isotopic ratio,  $R_X$  the isotopic ratio in the dissolved phase at any time during reaction, f the proportion of X remaining in the dissolved phase at that time in the reaction and  $\alpha$  the fractionation coefficient. Equation (2) can be rewritten in a form that is convenient for numerical computation:

$$\Delta \delta_X \approx \varepsilon \ln f \tag{3}$$

where  $\varepsilon = 1,000 \ (\alpha - 1)$  and  $\Delta \delta_X$  represents the change in the  $\delta^{13}$ C of the  $\Sigma$ CO<sub>2</sub> in solution. Thus,  $\Delta\delta_X$  versus ln f should have a slope  $\varepsilon$ . At any point in a Rayleigh distillation, the product material will differ from the remaining material by approximately  $\epsilon \%$ .

'Reverse distillation' (the release of CO<sub>2</sub> from organic matter to the dissolved phase) may follow the distillation line if the material utilized is released without fractionation. This condition need not apply, although it applies here to the carbon metabolism data.

In the case of calcifying organisms, such as corals, or calcifying communities, such as coral reefs, variations in  $\Sigma CO_2$  and  $\delta^{13}C$  of  $\Sigma CO_2$  reflect both calcification and organic metabolism. The  $\delta^{13}C$  of coral reef CaCO<sub>3</sub> is near the  $\delta^{13}C$  of  $\Sigma CO_2$  in seawater (0 to -5%; see, for example, ref. 11), whereas the  $\delta^{13}$ C of organic material from coral reefs is about -14 to -18% (ref. 12). Moreover, calcification ordinarily induces CO<sub>2</sub> changes substantially smaller than CO<sub>2</sub> changes from organic carbon metabolism<sup>6,8</sup>. We conclude that, as calcification is a weakly fractionating reaction, we can compare  $\delta^{13}C$  with calcificationcorrected  $\Sigma CO_2$  as an indicator of organic metabolism (calcification correction is  $\Delta$  total alkalinity/2)<sup>6,8,13,14</sup>.

Figure 1 describes the relationship between  $\Delta \delta^{13}$ C and calcification-corrected  $\Sigma CO_2$  (expressed as the fraction remaining), as determined by the aquarium experiments. Note that for CO<sub>2</sub> released during respiration, the data are described by the same regression relationship as the distillation, or uptake, curve. For this  $\delta^{13}$ C fractionation,  $\varepsilon$  is -18.6% ( $\sigma = 0.7\%$ ).

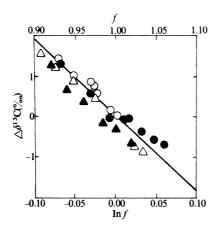


Fig. 1 Relationship between the change in  $\delta^{13}$ C ( $\Delta \delta^{13}$ C) and the fraction of calcification-corrected ΣCO<sub>2</sub> remaining {[CO<sub>2</sub>- $(\Delta \ alk/2)]/CO_2 \ initial\} \ in \ laboratory \ incubations \ of \ corals \ (light,\bigcirc;$ dark lacklose) and algae (light,  $\triangle$ ; dark, lacklose). The geometric regression line is  $\delta^{13}C = 0.04 - 18.6 \ln f$ .

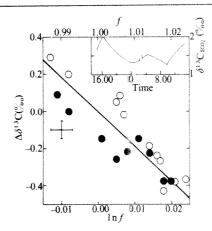


Fig. 2 Relationship between  $\Delta \delta^{i3}$ C and the fraction of calcification-corrected  $\Sigma CO_2$  remaining from field experiment on algal-coral reef community. Also included is the variation of the  $\delta^{13}$ C of the  $\Sigma$ CO<sub>2</sub> as a function of the time of day. The offset between midnight and 5 a.m. represents new water from outside the reef. Note the 5× scale expansion relative to Fig. 1. The cross represents the analytical precision of the measurements. The line through the data is that calculated for the laboratory experiments. O, Light; O, dark.

We offer the following independent estimate of the carbon isotopic fractionation coefficient. During our experiments, the seawater  $\Sigma CO_2$  had an average  $\delta^{13}C$  of  $\sim 2\%$ ; a fractionation of -19% would result in the tissue having a  $\delta^{13}$ C of about -17%. This is in agreement with observed values of -14 to -18% (ref. 12).  $\delta^{13}$ C can be routinely measured with a precision of  $\pm 0.03\%$ . The ΣCO<sub>2</sub> content of seawater is about 2,000 μmol l<sup>-1</sup>. The precision with which  $\Sigma CO_2$  changes can be inferred from isotopic changes is calculated from equation (3) to be about 3 umol 1

Figure 2 shows the data from the field experiment. The geometric regression slope derived from the field data (-18.8) is virtually identical with the laboratory data (-18.6). Daytime  $\delta^{13}$ C increases and night-time  $\delta^{13}$ C decreases average ~0.09%  $h^{-1}$ . Using  $\varepsilon = -19$  and the  $\Sigma CO_2$  of seawater = 2,000  $\mu$ mol  $l^{-1}$ , solving equation (3) gives an average rate of change of  $\Sigma CO_2$  of  $-9 \mu$ mol  $l^{-1} h^{-1}$  in the daytime and  $+9 \mu$ mol  $l^{-1} h^{-1}$  at night. Summing the daytime rate over 12 h and multiplying by 3 m water depth yields a daytime production rate for the entire water column of 3.9 g C m<sup>-2</sup> day<sup>-1</sup>. A 24-h summation for the night-time rate data yields a respiration rate of 7.8 g C per m<sup>2</sup> per day. Gross production is calculated as half the 24-h respiration plus the daytime production, or 7.8 g C per m<sup>2</sup> per day<sup>15</sup>. These figures are close to previous estimates<sup>6</sup> that coral-reef organic carbon production is 5-10 g C per m<sup>2</sup> per day.

We conclude that natural 13C isotopic changes can be used to calculate aquatic metabolic rates. We have measured the fractionation coefficient for this process by inducing relatively large composition changes in laboratory aquaria and then have validated our model with field data. It should be possible to apply this approach to a variety of biological communities.

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### Bacillus anthracis on Gruinard Island

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During the Second World War, trials of Bacillus anthracis as a potential agent of biological warfare (BW) were carried out on Gruinard Island off the west coast of Scotland (57°56' N. 5°35' W). Small bombs containing a slurry of spores of B. anthracis were detonated: most were suspended, about 6 feet above ground, from a gantry but one was dropped from an aircraft. The resulting aerosol clouds of spores were observed to pass through lines of sheep tethered at various distances downwind of the detonation site and after a few days several sheep died of anthrax. The lethal nature of BW weapons used in the open had, therefore, been shown; the unfortunate legacy was an island heavily contaminated with the persistent spores of a virulent microorganism. Soil samples were tested annually from 1948 to 1968 and again in 1972 by Ministry of Defence staff and were found to contain viable spores of B. anthracis (unpublished work), although accurate counts were not made. We report here the results of the first full survey of the extent of the contaminated area and the numbers of viable spores of B. anthracis present. The survey, carried out in 1979, shows that areas around the gantry remain contaminated to a detectable level but a much wider surrounding zone of undetectable contamination, possibly containing localized high concentrations of spores, could also constitute a hazard.

The whole island was marked out with a grid system (Fig. 1), soil core samples being taken at each marker by pushing a hollow tube (350 mm long  $\times$  25 mm internal diameter) into the ground to a maximum depth of  $\sim$ 300 mm. In the area close to the side of the gantry, 10 closely grouped cores were taken and a second similar group was collected  $\sim$ 50 m north west of the gantry in an area which had been traversed by the spore clouds. In the laboratory, the cores were expelled from their tubes into glass jars and shaken with twice their weight of 0.1 M phosphate buffer (pH 7.0) for 3 min on a 300 r.p.m. orbital shaker. The soil-buffer mixtures were heated at 60 °C for 1 h to destroy vegetative microorganisms, thus ensuring that only spores would be cultured. After allowing large particles to settle, 0.2 ml of the supernatant fluid was spread on each of five nutrient agar plates (Oxoid) which were then incubated at 37 °C for 24 h.

The growth of the natural aerobic spore-forming soil microflora was so profuse that it was necessary to dilute the soil extract at least 1 in 2 to distinguish colonies of B. anthracis, and consequently the presence of low numbers of B. anthracis spores would probably not have been detected. Colonies on nutrient agar which were suspected of being those of B. anthracis were isolated in pure culture and subjected to various confirmatory tests, phage sensitivity and the 'string of pearls test'2 which is based on the sensitivity of the organism to penicillin. The mouse LD<sub>50</sub> of isolates satisfying the confirmatory tests was determined by inoculating spores intraperitoneally in mice. A comparison between the Vollum laboratory strain (LD<sub>50</sub>, 49 spores; 95% confidence limits 20-91 spores) and isolates from the island (LD<sub>50</sub>, 115 spores; 95% confidence limits 38-270 spores) indicated that the latter had not lost their virulence for mice. Blood smears stained with polychrome methylene blue invariably showed the presence of the vegetative rods of B. anthracis, characteristically dark blue with a pink capsule. B. anthracis was detected in 20 out of 153 soil samples. All the positive samples were taken close to the gantry and at 50 m north west of the gantry; the numbers were small (Table 1) and we did not detect B. anthracis in any of the samples taken at the grid markers.

To confirm and extend the above technique we developed one that uses a selective medium and will detect as little as 3 spores per g of soil (Table 2). This involves mixing Gruinard soil with twice its weight of distilled water and agitating vigorously in a domestic blender for 5 min. The soil slurry was strained through a double layer of cotton gauze and 0.2-ml volumes were spread undiluted on the medium devised by Kniseley<sup>3</sup>, which allowed growth of colonies of *B. anthracis* but suppressed growth of the normal soil microflora. Phosphate buffer pH 7.2, water adjusted

## GRUINARD ISLAND Sampling positions 1979

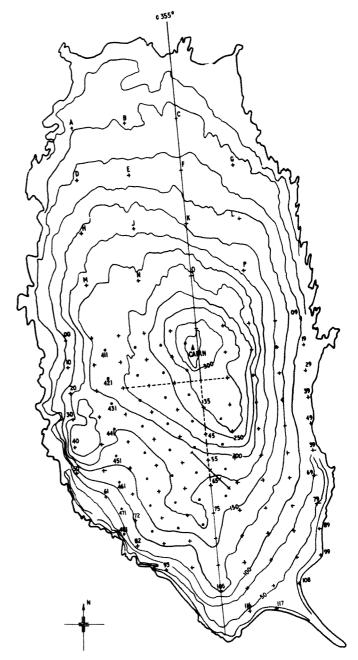


Fig. 1 The northern half of Gruinard Island was subdivided by a 200-m grid represented by the letters A-P, and the southern half by a 100-m grid numbered 00-117. Each cross represents a marker post. The southern grid was further subdivided by placing a marker post represented by ● at the intersections of the diagonals of the 100-m squares and the lines of these posts were numbered 411, 421, etc. The area where BW trials were carried out is indicated by the short line running NW-SE between markers 55 and 65. The gantry location is indicated by the intersect at the SE end of the line and the other intersects represent distances of 50, 100 and 200 yards downwind (scale 1: 10,000).

Table 1 Number of spores of B. anthracis detected per g of soil in samples close to the gantry and at 50 yards NW of the gantry

			Core no.		
Site	1	2	3	4	5
Gantry 50 yards NW	312 46	115 64	385 144	270 120	125 32

Table 2 Distribution of spores of B. anthracis with soil depth

Depth	Spore	e count pe	er core in	n each	Average spore count per g of	% At indicated
(cm)	1	2	3	4	soil	depth
0-2	270	1,110	810	180	68	43
2-4	18	189	1,704	540	71	43
4-6	99	54	293	33	12	13
6-8		30	29	0	2	1
8-14		0	0	0	0	0

Cores collected from the gantry area were sliced into sections 2 cm deep. Each slice was assayed individually for its content of spores of B. - Indicates that the core did not extend to that depth.

to pH values between 5 and 9, and an aqueous solution of 0.1% Tween 80 were also tried as extraction fluids, but no improvements were apparent compared with distilled water. In routine use one viable spore present in a 1-ml sample of soil extract could be successfully cultured. This represents 3 spores per g of soil, equivalent to  $3 \times 10^5$  spores per m<sup>2</sup> of ground. Using the new technique, four times more colonies of B. anthracis were observed than with nutrient agar, and at low levels of contamination (<12 spores per g) B. anthracis was detected by the new technique in samples which otherwise gave negative results.

One of the most important considerations was the distribution of spores with soil depth. Nearly all the viable spores of B. anthracis were found in the top 6 cm of soil (Table 2). This superficial distribution may reflect the slow accumulation of humus on the surface of the soil in this habitat which will delay the rate at which spores become buried.

It has been suggested that in suitable conditions spores of B. anthracis will germinate and proliferate<sup>4</sup>. The presence of small pools of water, neutral to slightly alkaline in reaction, containing decaying vegetation as a nutrient source, and warm weather might encourage the pathogen to multiply. It is our view that such conditions do not occur on Gruinard Island. The soil is acid (pH 4.2-4.7), the native flora slow to decay and the weather generally cool. We have shown that if soil cores are incubated at 37 or 22 °C the spores remain dormant and that spores prepared in the laboratory will not germinate on culture media prepared from extracts of Gruinard Island soil. Although  $\sim 4 \times 10^{14}$ spores were released during the BW trials, the recent surveys have revealed that only a small proportion remain near the surface of the soil. To simulate conditions in which multiplication of these remaining spores might occur, such as the

Table 3 Effect of additions of nutrients on spore counts of B. anthracis

		Incubation	Spore cou	nt per core
Core no.	Nutrient	temperature (°C)	Initial inoculum	Final
1	Blood	37	$5 \times 10^2$	$3.3 \times 10^{9}$
2	Blood	22	$5 \times 10^2$	$3.5 \times 10^{5}$
3	Faeces	37	$5 \times 10^2$	$1.7 \times 10^{3}$
4	Faeces	22	$5 \times 10^2$	$5.7 \times 10^{2}$
5	None	37	$5 \times 10^2$	$3.9 \times 10^{2}$
6	None	22	$5 \times 10^2$	$6.6\times10^2$

Cores (~100 g) from an uncontaminated area close to the gantry were inoculated with about 500 spores of B. anthracis (Vollum) incubated with the indicated nutrient, 5 ml of calf blood or 5 g of rabbit faecal pellets, and assayed for their content of spores of B. anthracis after 7 days' incubation.

nutrient body fluids from a dead animal soaking into the soil, calf blood (5 ml) was added to anthrax-containing soil cores (~50 g) which were then incubated for 7 days at 37 or 22 °C. Addition of blood raised the pH of the soil from 4.5 to 7.2 and after incubation at either temperature these cores showed a large increase in their spore count (Table 3). Other cores supplemented with faecal pellets from rabbits demonstrated a small increase in count only after incubation at 37 °C. It is apparent, therefore, that death of an animal in a contaminated area could provide a favourable growth environment for the proliferation of B. anthracis, and a short period of warm weather would be sufficient to initiate germination followed by growth and formation of a high concentration of second generation spores at the surface of the soil. Such a combination of circumstances would probably be rare but could produce localized high concentrations of the organism close to the soil surface: these have not been found.

Thus, we have developed a technique which will detect B. anthracis spores in Gruinard Island soil at concentrations as low as 3 spores per g of sample, and further work would enable us to define the area contaminated to this level. Nevertheless, it is likely that an area of undetectable contamination would surround such an area. The technique could also be applicable to the examination of soil samples from other localities.

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# Regional accumulation of vegetal pole poly(A)+ RNA injected into fertilized Xenopus eggs

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Cytoplasmic determinants localized in particular regions of the egg seem to be important in cell determination during early embryogenesis<sup>1,2</sup>. Although their composition is uncertain, there is evidence that some are partly composed of maternal RNA molecules<sup>3-6</sup>. Consistent with this possibility, maternal poly(A) RNA and certain specific RNA sequences are reported to show an uneven distribution in the cytoplasm of oocytes and developing embryos<sup>7-11</sup>, suggesting that maternal RNA sequences may contain signals required to distribute themselves in specific cytoplasmic regions of the egg. We have now tested this idea by analysing the distribution of RNA sequences localized in the vegetal pole cytoplasm of Xenopus laevis eggs after microinjection into fertilized zygotes. We report that exogenous vegetal pole poly(A)+ RNA accumulates along a concentration gradient from the vegetal hemisphere to the animal hemisphere of the injected egg and provide evidence that the concentration gradient is set up by active migration of the injected vegetal pole poly(A) + RNA into the vegetal hemisphere cytoplasm between fertilization and the first cleavage.

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Ovulated eggs were orientated and sectioned with a cryotome into six regions through planes perpendicular to the animal-vegetal axis. The sections containing the polar regions of the egg were pooled into animal and vegetal pole groups, their RNA was extracted and labelled *in vitro* by RNA ligase-catalysed transfer of <sup>32</sup>P-cytidine-3',5'-diphosphate to the 3' termini. The labelled RNA was separated into poly(A)<sup>+</sup> and poly(A)<sup>-</sup> fractions and injected into either the animal or the vegetal hemisphere of fertilized eggs ~15-20 min after insemination. The injected embryos were incubated at 18 °C until they reached the proper developmental stage. They were then fixed, processed for histological examination and the distribution of labelled RNA along their animal-vegetal axes was determined by autoradiography.

To ensure that the 3' label was not transferred from the injected RNA to another molecular species, some of the histological sections were treated with enzymes before autoradiography. As shown in Table 1, the number of grains developed in the autoradiographs was not markedly affected by DNase treatment. Grain development was severely decreased,

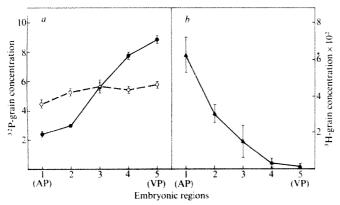


Fig. 1 Grain distribution along the animal-vegetal axis of four-cell embryos after injection of various RNA fractions. Groups of 75-100 dejellied ovulated eggs suspended in a solution containing 20 mM Tris-HCl pH 7.5, 25 mM KCl, 15 mM MgCl<sub>2</sub>, 5% sucrose and 0.1% Triton X-100 (Solution A) were oriented animal hemisphere up, frozen in a dry iceacetone mixture and sectioned with a cryotome into six 200-µm regions perpendicular to the animal-vegetal axis according to the procedure of Moen and Namenwirth<sup>15</sup>. The animal and vegetal pole sections were collected, pooled into separate groups and homogenized (five strokes up and down of a Potter-Elvehjem glass homogenizer with a teflon pestle) in 3 vol of Solution A containing  $15~\mu g\,ml^{-1}$  sodium heparin (4 °C). The homogenate was centrifuged at 10,000g for 10 min at 4 °C, the supernatant fraction was collected and adjusted to 0.5% SDS and to 100 mM with Tris-HCl pH  $9.0^{16}$ RNA was extracted by shaking the suspension with phenyl/chloro-form/isoamyl alcohol (50:50:1). The deproteinized RNA was ethanol precipitated, washed and resuspended (420 µg ml<sup>-1</sup>) in a solution containing precipitated, washed and resuspended (420 µg m<sup>-</sup>/<sub>1</sub> m<sup>-</sup>/<sub>2</sub> solution containing 50 mM Tris-HCl pH 7.5, 10 mM MgCl<sub>2</sub>, 1.5 mM ATP, 6.6 mM dithiothreitol, 1.28 Ci ml<sup>-132</sup>P-cytidine-3', 5'-diphosphate(NeN), and ~230 U ml<sup>-1</sup> of RNA ligase (EC,6.5.1.3, New England Biolabs). The mixture was incubated at 4 °C for 16 h to transfer the labelled nucleotide to the 3' terminus of the RNA substrate<sup>17</sup>. The efficiency of labelling and the integrity terminus of the RNAs upoduct. The elitative of the labelled RNA product was checked by sucrose density gradient centrifugation. RNA labelled to a specific activity of  $\sim 3 \times 10^3$  d.p.m.  $\mu g^{-1}$ was separated into poly(A)+ and poly(A)- fractions by oligo(dT)-cellulose chromatography<sup>18</sup>, ethanol precipitated, dissolved in the microinjection buffer<sup>19</sup> and injected in 30-nl aliquots into the animal or vegetal hemisphere of fertilized eggs (15-20 min after insemination) by the method of Gurdon<sup>19</sup>. The injected volume contained 5-15 ng of poly(A)<sup>-</sup> RNA or the entire oligo(dT)-cellulose-eluted fraction (poly(A)<sup>+</sup> RNA) derived from 5-15 ng of total RNA. Taking into account the specific activity of the injected RNA. the number of grains detected in a section cut through a diameter of the one-cell zygote, and the efficiency of autoradiography, we calculate that ~95% of the injected RNA radioactivity entered the egg. Injected embryos were allowed to develop at 18 °C until the four-cell stage, when they were fixed with Bouins and embedded in paraplast. Sections were cut at 13 µm, extracted with cold 5% trichloroacetic acid, coated with undiluted NTB-2 liquid emulsion (Eastman-Kodak, New York) and exposed for 2 months The developed slides were stained with Harris haematoxylin and examined by dark-field microscopy to avoid confusing the grains with pigment granules<sup>10</sup>. The grains were counted in 400-µm<sup>2</sup> areas spaced at equidistant intervals (usually ~240 µm, but were varied according to the diameter of the embryo). a, O, Represent the distribution of the injected vegetal pole poly and • the distribution of the injected vegetal pole poly(A)+ RNA; b, ▲ represent the distribution of injected 3H-polyuridylic acid (NEN). Animal (AP) and vegetal (VP) regions of the embryo are to the left and right respectively.

however, when the sections were treated with RNase in conditions which hydrolyse both RNA and its poly(A) sequence<sup>12</sup>. Thus the label remains in RNA after injection.

Figure 1 shows the distribution of grains along the animalvegetal axis of four cell embryos injected with various RNA fractions after fertilization. When vegetal pole poly(A)<sup>+</sup> RNA was injected, it appeared to accumulate in a gradient from the vegetal hemisphere to the animal hemisphere with four to five times more grain density in the region nearest the vegetal pole than near the animal pole. Such vegetal to animal hemisphere gradients were observed regardless of whether the vegetal pole poly(A)+ RNA was injected into the animal or vegetal hemisphere, suggesting that the entry point on the egg surface was unrelated to the distribution of the exogenous RNA sequences in the cytoplasm. No significant difference in grain distribution was seen along the other primary axes of the embryos. Two control injections were carried out in parallel with the injection of vegetal pole poly(A)+ RNA. Labelled polyuridylic acid, whether introduced into the animal or the vegetal hemisphere, became distributed along a steep gradient of opposite polarity to that exhibited by the vegetal pole poly(A)+ RNA (Fig. 1), suggesting that the microinjection procedure itself does not dictate the pattern of exogenous RNA distribution. Vegetal pole poly(A) RNA was also injected into fertilized eggs and, in contrast to the poly(A)+ RNA, it showed a relatively homogenous distribution along the animal-vegetal axis of four-cell embryos (Fig. 1).

The results of the vegetal pole poly(A)<sup>+</sup> RNA injections do not necessarily imply that this RNA fraction accumulates along a vegetal to animal hemisphere gradient in a specific fashion. Polyadenylic acid accumulates in a similar gradient after injection into fertilized *Xenopus* eggs<sup>13</sup>, and it is conceivable that the poly(A)+ RNA passively accumulates in the vegetal hemisphere cytoplasm, possibly because of an adventitous association with yolk platelets. To resolve this issue, we injected labelled poly(A)+ RNA from the animal pole region of the ovulated egg into fertilized eggs and analysed its distribution in four-cell embryos. Unlike the vegetal pole poly(A)<sup>+</sup> RNA, the animal pole poly(A)+ RNA did not accumulate primarily in the vegetal hemisphere cytoplasm, but tended to concentrate in the animal hemisphere cytoplasm (Table 2). Others have shown that injected poly(A)<sup>+</sup> globin mRNA also accumulates along a gradient from the animal to the vegetal hemisphere blastomeres in Xenopus embryos 13. These results suggest that the accumulation of vegetal pole poly(A)+ RNA in a vegetal to animal hemisphere gradient is a specific property of the RNA sequences from the vegetal pole region of the egg.

There are three possible explanations for our results: the injected molecules may initially be distributed evenly in the egg and subsequently degraded in the animal hemisphere cytoplasm by localized nucleases; or the injected RNA molecules may be preferentially segregated into the vegetal hemisphere blastomeres by cytoplasmic movements accompanying the second cleavage; or the observed pattern of vegetal pole poly(A)<sup>+</sup> RNA distribution may be due to selective interaction of the injected RNA with specific recognition sites enriched in the vegetal hemisphere regions of the uncleaved zygote and maintained in the four-cell embryo. If region-specific RNA turnover occurs in

Table 1 The effect of DNase and RNase treatment on grain development in embryos microinjected with labelled RNA

Treatment	Grain concentration ± s.d.	% Of control
None	$11.0 \pm 1.4$	
DNase	$10.0 \pm 0.7$	90.9
RNase	$2.1\pm1.4$	18.2

Sections were treated for  $16\,h$  with  $50\,\mu g\,ml^{-1}$  bovine pancreatic DNase I(EC 3.1.4.5) dissolved in  $100\,mM$  Tris-HCl pH 7.5, 3 mM MgCl<sub>2</sub> or  $50\,\mu g\,ml^{-1}$  bovine pancreatic RNase A(EC 3.1.4.22) in  $10\,mM$  Tris-HCl pH 7.5,  $10\,mM$  NaCl,  $1\,mM$  MgCl<sub>2</sub> (refs 8, 12) at 37 °C. Grain counts were made in 400- $\mu m^2$  regions in the central areas of four cell embryos.

Table 2 Grain distribution in the animal and vegetal hemispheres of developing embryos injected with poly(A)+ RNA

			Grain o	concentration	±s.d.
RNA source	Developmental stage	Sample size	Animal	Vegetal	Vegetal/ animal ratio
Animal pole	4 cell	3	$18.0 \pm 1.7$	$9.3 \pm 3.0$	0.5
Vegetal pole	1 cell	4	$20.6 \pm 2.0$	$62.4 \pm 2.4$	3.0
Vegetal pole	4 cell	6	$17.1 \pm 1.3$	$43.8 \pm 1.3$	2.6
Vegetal pole	Blastula	8	$14.6 \pm 3.2$	$23.7 \pm 4.3$	1.6

Animal and vegetal pole  $poly(A)^+RNA$  was derived from different eggs. In the vegetal pole  $poly(A)^+RNA$  experiment, clutches of inseminated eggs were injected with equal amounts of RNA, allowed to develop for the desired time at 18 °C, fixed and processed for histology and autoradiography. Grains were counted in a 400-µm<sup>2</sup> area in the centre of each hemisphere.

the animal hemisphere cytoplasm, we might expect the distribution of injected vegetal pole poly(A)+ RNA between the animal and the vegetal hemisphere regions to be more uniform before the four-cell stage. This possibility was tested by injecting equal amounts of the vegetal pole poly(A)+ RNA into a clutch of fertilized eggs and examining its distribution in embryos killed at various times during early embryogenesis. The accumulation of vegetal pole poly(A)+ RNA in the vegetal hemisphere was more pronounced in the one cell zygote than in the four-cell embryo or the blastula (Table 2), suggesting that the injected RNA is gradually degraded or modified in the vegetal rather than the animal blastomeres during early embryogenesis, perhaps in concert with the relatively high rate of protein synthesis known to occur in the vegetal cells<sup>14</sup>. These results do not support a role for selective degradation of the vegetal hemisphere accumulation of injected vegetal pole poly(A)+ RNA. The unequal distribution of vegetal pole poly(A)+ RNA as early as 20-30 min after injection, when the embryo is still a single cell (Table 2), is also inconsistent with differential segregation to the vegetal blastomeres during the second cleavage.

The formation of a vegetal to animal pole gradient in fertilized zygotes by poly(A)+ RNA from the vegetal pole of unfertilized Xenopus eggs is most simply explained by active migration. Although the possibility that some of the labelled termini of the injected poly(A)+ RNA are exchanged with endogeneous poly(A)+ RNA cannot be excluded, it is highly unlikely that this alone could lead to the observed patterns of labelled RNA.

Our results suggest that vegetal pole poly(A)\* RNA molecules may recognize specific cytoplasmic binding sites which are distributed along a concentration gradient from the vegetal to the animal pole of the egg. These sites could include cytoskeletal elements, membrane systems, specific types of yolk particles or other cytoplasmic organelles. It is not known whether information in the RNA sequence or in endogenous protein that may form complexes with the injected RNA molecules is responsible for this interaction. The ability of maternal RNA molecules or their native ribonucleoprotein complexes to recognize specific regions of the egg is consistent with their being cytoplasmic determinants.

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# Sodium and potassium channels in demyelinated and remyelinated mammalian nerve

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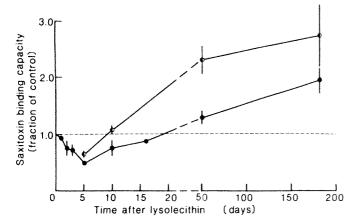
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Demyelination of peripheral axons initially causes failure of action potential conduction, probably because the internodal membrane lacks sodium channels 1.2. However, Bostock and Sears<sup>3</sup> showed that 3-14 days after demyelination of rat nerve with diphtheria toxin, some axons develop regenerative inward currents in the internodal membrane permitting continuous (non-saltatory) conduction. In nerves that have remyelinated or regenerated, the number of nodes per unit length of fibre characteristically increases greatly<sup>4,5</sup>. As the internodal membrane does not normally possess sodium channels<sup>1,2</sup>, both the earlier appearance of continuous conduction in the demyelinated nerve and the later presence of extra nodes in the remyelinated nerve require either a laying down of new sodium channels or a redistribution of the sodium channels in the original nodes. The present experiments examine the changes in the total number of sodium channels, measured by saxitoxinbinding capacity<sup>1</sup>, that occur in rabbit sciatic nerves which have been demyelinated in vivo with lysolecithin2.5 and then allowed to remyelinate. The results provide no evidence for the formation of new sodium channels during the early stage, when continuous conduction may develop, but show clearly that new channels are formed during remyelination.

Sciatic nerves of rabbits under methoxyflurane anaesthesia were injected on one side in the mid-thigh (20-30 mm distal to the sciatic notch) with lysolecithin (40 µl of a 1% solution under the sheath) and allowed to recover. Reflex function on the affected side was markedly depressed for the first 1-2 weeks, but thereafter recovered. At various times afterwards (1-200 days, see Fig. 1 legend) the rabbits were killed by injection of air into an ear vein, and from each animal both the lysolecithin-treated and control nerves were removed and desheathed. The sodium channel density in 25-30-mm lengths corresponding to the injected region was determined in both the treated and control homogenized nerves from measurement of the saxitoxin-binding capacity<sup>1</sup>. The specific activity of the saxitoxin was initially 7 d.p.m. fmol<sup>-1</sup>, and its radiochemical purity 68%. The mean uptake for the series, 8.4 fmol per mg wet weight, calculated on the basis of the initial purity, is about half that described previously1, because over the several months of these experiments the specific activity declined substantially through backexchange of the label into the water of the solution<sup>1</sup>. Errors from this source, however, were avoided by expressing the uptake by remyelinated nerve relative to that of the control nerve determined at the same time and in identical conditions. Uptakes were usually determined from a labelled saxitoxin concentration of 5 nM, about four times the value of the equilibrium dissociation constant (K) for saxitoxin in this homogenized nerve1. Some experiments, done with 20 nM saxitoxin, gave uptakes about the same as with 5 nM for both normal and lysolecithintreated nerves, confirming that any change in the value of K would not have significantly affected the results.

Histological examination of two nerves at 7 weeks indicated that a large proportion (~70%) of the fibres had remyelinated (and so presumably had originally been demyelinated), as



judged by the observation of single axons, each within its own Schwann cell tube, that were smaller in diameter and more thinly ensheathed with myelin than surrounding normal fibres and those in control nerves; in six nerves examined after 7-10 days there were clear signs of demyelination but little or none of degeneration. It would have been interesting to determine the total number of nodes in the tissue at these times. However, this formidable task was not undertaken, and, unfortunately, no such information is available in the literature. In regenerated rabbit nerve, however, where short internodes are found (at least up to 1 yr after injury), Cragg and Thomas<sup>4</sup> have shown that the average number of nodes per unit length increases 1.4 times for a 10-µm diameter fibre, and almost doubles for the largest (21-22 µm diameter) fibres. Remyelination is known to lead to a similar decrease in internodal length<sup>6</sup> and so, presumably, to a corresponding increase in the number of nodes per unit length.

Initially, the saxitoxin-binding capacity of the lysolecithintreated nerves (expressed per unit weight of desheathed nerve) decreased relative to that of the contralateral controls, but after 2-6 months the binding capacity increased above its control value (Fig. 1). However, the results as expressed in Fig. 1, comparing the binding per unit weight, are distorted by the connective tissue hyperplasia and oedema that follow intraneural injections; and much more significant effects are seen when comparison is made on the basis of binding per unit length as was done in many of the experiments. For example, 5 days after the lysolecithin injection, the weight per unit length of the injected nerve was  $42 \pm 5\%$  greater than in its contralateral control. Thus, expressed per unit weight of nerve, the saxitoxinbinding capacity at this time had fallen to  $0.48 \pm 0.04$  (n = 6), whereas, expressed per unit length of nerve, the fall was only to  $0.65 \pm 0.06$ . Similarly, when saline instead of lysolecithin was injected, after 5 days the saxitoxin-binding capacity expressed per unit weight fell to  $0.77 \pm 0.10$  (n = 4) whereas, expressed per unit length, it remained roughly constant at  $0.96 \pm 0.10$ . However, ligation of a normal nerve ~1 cm distal to the sciatic notch, which involves less surgical interference in the region of the more distal usual injection site, led after 5 days to a fall in channel density in the latter portion of the degenerating nerve that was about the same whether expressed per unit weight or per unit length (to final values of  $0.31 \pm 0.05$ , n = 4, and  $0.33 \pm$ 0.07, respectively). Histological examination of two salineinjected nerves after 5 days showed no grossly abnormal morphology of the myelinated fibres.

The early decrease in binding is difficult to interpret without quantitative information about the amount of fibre degeneration caused by the lysolecithin injection, which the experiments on ligated nerves showed would lead to a rapid decrease in toxin binding. Histological examination showed that little degeneration occurred. Because, in principle, the amount of degeneration might be assessed by measuring binding in distal segments of the nerve sufficiently far down to escape any 'spill-over' demyelination, saxitoxin binding was determined in many experiments in regions both proximal and distal to the injected region. In the region proximal to the lesion in 26 nerves examined 5 days-2 months after injection, the weight per unit length on the injected side and the saxitoxin uptake per unit weight were not significantly different from their control values  $(1.02 \pm 0.05)$  and  $1.04 \pm 0.10$ , respectively). However, in the distal segment the corresponding values were  $1.30 \pm 0.09$  and  $0.95 \pm 0.10$  (n = 17), respectively, showing that some spill-over of the effect distally did indeed occur. Even when the portion of the nerve between the knee and the ankle was examined—a region 40-80 mm from the usual site of injection—there was still an increased binding of saxitoxin (per unit length) of  $47 \pm 13\%$  (compared with a value of  $79 \pm 26\%$  around the injection site) in five nerves that had been injected 7 weeks previously. The morphological basis for this increase remains unclear.

In nerves examined about 2 months after lysolecithin injection, when remyelination was well under way (although not complete because the myelin sheaths were still relatively thin), the weight per unit length was observed to be  $65 \pm 8\%$  greater than in the control. The value of  $1.30 \pm 0.13$  for the channel density of remyelinated nerves shown in Fig. 1 for this time thus corresponds to a value for the sodium channel density per unit length of remyelinated nerve of  $2.31 \pm 0.24$  (n = 13) that of the control. These experiments, therefore, do not support the idea that the source of sodium channels for the extra number of nodes that appear on remyelination is derived from the population of sodium channels in the original nodes. Rather, there is an increased laying down of extra sodium channels in the membrane, consistent with the increased number of nodes, as if the total number of channels per node remains relatively constant.

In some experiments the nerves were mounted in a capillary chamber containing a series of annular platinum electrodes for electrical recording of the monophasic compound action potential, and exposed for 10-15 min to the potassium channel blocking agent 4-aminopyridine. In frog sciatic nerve such treatment leads to a marked increase in the duration of the compound action potential because the blockage of the repolarizing potassium current leads to a broadening of the individual action potentials in each individual fibre (Fig. 2a). However, in normal rabbit sciatic nerve, as already observed in normal rat nerve7, there is at most only a slight broadening of the compound action potential (Fig. 2c, e), presumably reflecting the normal absence of potassium currents<sup>8,9</sup> in all but a small fraction of the fibres. However, after treatment with lysolecithin, rabbit sciatic nerves acquire a distinct sensitivity to 4-aminopyridine (Fig. 2 b, d, f). In five experiments at 7 weeks and three at 6 months the average broadening at half-amplitude, which was  $15.6 \pm 3.5\%$  and  $29.5 \pm 5.1\%$ , respectively, was significantly larger than the broadening of  $3.5 \pm 1.6\%$  (n = 12) of control nerves (P < 0.001). In four experiments at 10 days the broadening of  $7.8 \pm 3.5\%$  was not significant (P > 0.2).

As illustrated in Fig. 2, the compound action potential in the lysolecithin-treated nerve was consistently wider than in the contralateral control; for example, at 7 weeks the action potential was  $47.4 \pm 17.8\%$  broader and conducted  $27.2 \pm 11.0\%$  (n = 5) more slowly. This indicates that the electrophysiological characteristics of the remyelinated axons differ from those of the normal nerve; however, these differences have not been analysed in more detail.

These findings indicate that not only do new sodium channels appear in the remyelinated nodes but also that in the remyelinated nerve, unlike normal nerve (at least 7 weeks-6 months

after demyelination), potassium channels carry part of the current during the action potential. Whether or not these potassium channels are present in newly formed nodes remains unclear, for Sherratt et al. have shown that some rat nodes of Ranvier show signs of paranodal demyelination a few days after diphtheria toxin and are sensitive to 4-aminopyridine. Therefore, one explanation for the sensitivity to 4-aminopyridine in the present experiments would be that the lysolecithin had initially caused a paranodal demyelination of some existing nodes, thus exposing pre-existing paranodal potassium channels, and that it was the 4-aminopyridine sensitivity of persisting damaged original

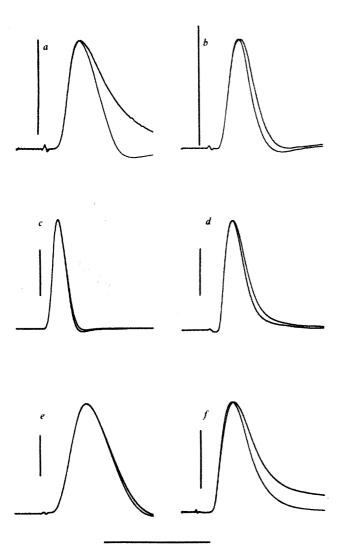


Fig. 2 The effect of 4-aminopyridine on the compound action potential of frog (a) and rabbit (b-f) desheathed sciatic nerves. Each trace consists of a record taken after about 10 min exposure to 4-aminopyridine (200-2,000 µM) superimposed on the control record before exposure; in all pairs the action potential with the longer duration occurs in the treated nerve. Record a is from a frog nerve at 20 °C. Records c and d are from the right and left sciatic nerves, at 37 °C, of a 28-week-old rabbit, whose left sciatic nerve had been injected 7 weeks previously with 40 µl lysolecithin. Records e and f are the corresponding control and treated nerves. at 20 °C, from a 12-month-old rabbit whose left sciatic nerve had been injected with 40 µl lysolecithin 6 months previously. Record b shows another example of the broadening by 4-aminopyridine of the compound action potential, at 37 °C, of the sciatic nerve of a 28-week-old rabbit injected 2 months previously with lysolecithin. The vertical bar in each record represents 1 mV for the control record; the record in 4-aminopyridine has sometimes been scaled downwards slightly so that its amplitude is the same as that of the control to allow the shapes of the action potential to be more easily compared. The horizontal bar represents 1 ms for panels a-e and 2 ms for panel f.

nodes, rather than new nodes, that was observed in the present experiments. Note, however, that for this to account for the results of Fig. 2, any paranodal demyelination existing 7 weeks-6 months after the original exposure to lysolecithin would have to have persisted longer than is generally the case after lysolecithin in other mammals  $^{30-12}$ .

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## Receptor binding of somatostatin-28 is tissue specific

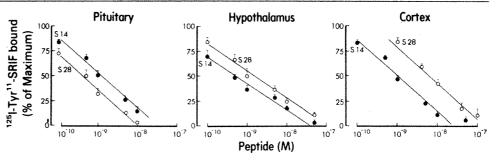
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Although somatostatin was originally identified in the hypothalamus as a tetradecapeptide (SRIF or S-14)1, subsequent studies have revealed that tissue somatostatin is heterogeneous comprising in addition to S-14 two larger forms with molecular wieghts (M<sub>r</sub>s) of 10,000-15,000 and 3,000 (refs 2-7). Recently a 28-amino acid peptide (somatostatin-28, S-28)-a 14-amino acid N-terminal extension of S-14-has been isolated from mammalian gut and hypothalamus<sup>8-10</sup>. Synthetic S-28  $(M_r, 3,160)$  has been shown to correspond to the 3,000- $M_r$  SLI species found in most tissues<sup>5</sup> and to exhibit greater potency than S-14 for inhibiting both endecrine (growth hormone, insulin, glucagon)<sup>11,12</sup> and exocrine (pancreatic enzymes and bicarbonate) secretion<sup>13</sup>. It is not clear whether S-28 exerts similar effects to S-14 on central nervous system functions<sup>14</sup> nor whether the S-14-like effects of S-28 are mediated through its conversion to S-14 or through direct action on the recently characterized S-14 receptors of rat brain and pituitary membranes<sup>15,16</sup>. We suggest here that the greater potency of S-28 for inhibiting growth hormone secretion is because it binds to pituitary S-14 receptors with 3.2-fold higher affinity than does S-14. Furthermore in the central nervous system, S-28 has a lower affinity than S-14 for the receptors, indicating that it is less potent than S-14 in regulating brain functions. Receptor binding of S-28 in both pituitary and brain occurred directly without significant conversion to S-14, suggesting that S-28 is a true S-14 receptor agonist but possesses distinct tissue specificities.

Membrane fractions enriched in somatostatin receptors were prepared from fresh cerebrocortical, hypothalamic and pituitary tissue obtained from adult male Sprague-Dawley rats using techniques reported previously 13-18. Tyr 11-SRIF was radioiodinated to high specific activity2 and used as the radioligand. Figure 1 depicts the specific binding of <sup>125</sup>I-Tyr<sup>11</sup>-SRIF to S-14 receptors in the pituitary, hypothalamus and cerebral

Fig. 1 Inhibition by S-28 and S-14 of the specific binding of <sup>125</sup>I-Tyr<sup>11</sup>-SRIF to membrane S-14 receptors in the pituitary, hypothalamus and cerebral cortex. Equilibrium binding studies were carried out as described previously  $^{15-18}$  by incubating 50  $\mu g$  membrane protein and  $5\times 10^{-11}$  M  $^{125}$ I-Tyr $^{11}$ -SRIF at 30 °C (40 min for brain membranes and 20 min for pituitary membranes) in 50 mM HEPES-KOH buffer, pH 7.5, containing bovine serum albumin



(BSA, 10 mg ml<sup>-1</sup>), MgCl<sub>2</sub> (5 mM), Trasylol (200 KIU ml<sup>-1</sup>), bacitracin (0.02 µg ml<sup>-1</sup>) and phenylmethylsulphonyl fluoride (0.02 µg ml<sup>-1</sup>). Receptor-bound radioligand was separated by centrifugation of the incubation mixture, and was washed in HEPES-KOH buffer containing BSA  $(10 \text{ mg ml}^{-1})$  and quantified in a  $\gamma$  spectrometer. The specific binding at each concentration was calculated by subtracting the nonspecific binding observed at high concentration of S-14  $(1 \times 10^{-7} \text{ M})$  from the total binding. To check the parallelism between inhibition curves of the two peptides, the residual variances of the two curves were tested for homogeneity by an f-test and the lines tested for parallelism by a t-test, both with the aid of the computer program of Faden and Rodbard<sup>2</sup>

cortex. Both S-14 and S-28 produced dose-dependent inhibition of radioligand binding over a concentration range of 10<sup>-1</sup> 10<sup>-7</sup> M. In each tissue, displacement curves obtained with the two peptides were parallel. The potency of S-28 relative to S-14 was calculated from the concentrations of the two peptides required to inhibit 125I-Tyr11-SRIF binding to the membrane receptors by 50%, as shown in Table 1. S-28 exhibited 3.2-fold greater affinity for binding to S-14 receptors in the pituitary, whereas in the hypothalamus and cortex, it exhibited three- and sixfold lower affinities respectively. To determine whether S-28 is converted to S-14 during incubation with the membrane receptors, we analysed the S-28-containing reaction mixtures at equilibrium by Sephadex G-25 (superfine) gel chromatography and radioimmunoassay for somatostatin using an antibody (R149)<sup>2</sup> reacting 100% with S-28 and S-14. In these conditions, >99% of the immunoreactivity emerging from the columns co-eluted with synthetic S-28, showing that S-28 is not converted to S-14 during incubation with membrane receptors.

These data suggest that the higher potency of S-28 for in vitro growth hormone inhibition is due to its greater affinity for binding to pituitary S-14 receptors, as the receptor-binding affinity of the peptide (Table 1) correlates well with its reported 3-14-fold higher potency for growth hormone suppression from cultured rat anterior pituitary cells11. Although S-14 is known to exert characteristic effects in the central nervous system14, the effects of S-28 on brain have not yet been characterized. Based on our brain receptor binding data, however, S-28 would be expected to show reduced S-14-like biological activity in brain. Because S-28 can be enzymatically cleaved to S-14 (ref. 19), and as breakdown of S-28 to the tetradecapeptide form could influence the comparison of the potency of \$-28 relative to \$-14, it was necessary to determine the extent of the conversion during our incubation studies. Our finding that S-28 remains intact in the experimental binding conditions indicates that this molecule possesses its own intrinsic receptor binding properties.

What is the physiological significance of S-28? The present data clearly suggest that it is not simply a superactive form of

Concentrations (IC50) of S-14 and S-28 required for 50% inhibition of  $^{125}\text{I-Tyr}^{11}\text{-SRIF}$  binding

	IC <sub>50</sub>	Relative potency				
Tissue	S-14	S-28	of S-28*			
Cortex Hypothalamus Pituitary	$0.9 \pm 0.12$ $0.5 \pm 0.15$ $1.25 \pm 0.22$	$6.2 \pm 0.11$ $1.5 \pm 0.03$ $0.41 \pm 0.014$	0.15 (0.07-0.2) 0.33 (0.26-0.61) 3.15 (1.98-10.9)			

IC<sub>50</sub> values are mean  $\pm$  s.e.m.; n = 6 experiments in duplicate. Values represent the mean and range of potencies of (compared with that of S-14 taken as 1) for the six experiments.

S-14. As S-28 (or equivalent immunoreactivity) is present in high concentrations in the hypothalamus (approximately one-third of the amount of S-14)<sup>20</sup> and seems to be released into the hypophysial portal circulation<sup>21</sup>, it (together with S-14) is likely to be a regulator of pituitary growth hormone secretion. Likewise, the predominance of the S-28 form of somatostatin in some areas of the intestine<sup>5</sup> suggests specific regulatory roles for this peptide in this organ. Whether the effects of S-28 are a manifestation of its interaction with S-14 receptors alone or whether there are specific S-28 receptors distinct from those of S-14 remains to be established. The reduced ability of S-28 to compete with 125 I-Tyr11-SRIF for binding to brain S-14 receptors observed here, together with the recent finding that S-28 competes better with a radioiodinated S-28 analogue (125I-[Leu<sup>8</sup>, D-Trp<sup>22</sup>, Tyr<sup>25</sup>]S-28) for binding to brain membranes<sup>22</sup> than does S-14, provide evidence for the existence of separate receptors for S-14 and S-28 at least in brain. In any case, our observation that S-28 exhibits receptor-binding activities distinct from those of S-14, the reported differences in the biological actions of the two peptides and the high concentrations of S-28 in some tissues suggest that this molecule not only serves as a precursor of S-14 but also possesses independent physiological functions.

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## Soluble factor activation of human B lymphocytes

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Medium conditioned by lectin (and/or antigen)-stimulated human peripheral blood lymphocytes has been shown to contain factors, termed interleukin 1 (IL-1) and interleukin 2 (IL-2)1. which augment and maintain human T-cell proliferation3respectively. Interleukin 2 (T-cell growth factor, TCGF) requires the presence of a macrophage, or its monokine product, IL-1, for its production by T cells<sup>7-9</sup>. It has been shown that multiple signals are required for stimulating both T- and B-cell proliferation<sup>8-10</sup> but the factors necessary for optimal B-cell mitogenesis have not been rigorously defined. As both B- and T-cell stimulatory agents are present in lectin-stimulated peripheral blood lymphocyte-conditioned media, we studied the effects of the interleukin factors on human B cells. Human peripheral blood B lymphocytes and pokeweed mitogen (PWM)-stimulated B-cell blasts were evaluated for their proliferative response to soluble growth factors, which were obtained from either lipopolysaccharide or phytohaemagglutinin (PHA)stimulated peripheral blood mononuclear cells. We show here that factors from lipopolysaccharide-stimulated conditioned medium were unable to stimulate initial B-cell proliferation. However, factors from PHA-stimulated conditioned medium stimulated <sup>3</sup>H-thymidine (TdR) incorporation in both B cells and lectin-activated T cells. This stimulation could be maintained for multiple re-exposures to the growth factors, which suggests that a soluble factor present in medium conditioned by lectin-stimulated peripheral blood mononuclear cells may modulate B-cell proliferation in a manner analogous to T-cell

Human interleukin 1 was purified from mononuclear cellconditioned media by diafiltration, ultrafiltration and isoelectric focusing (pH 6.8-7.2)11. This material was co-mitogenic for human thymocytes and human T cells at a concentration < 1 ng per 25 µl and could not support the continuous growth of cultured T cells<sup>12</sup>. Interleukin 2 preparations, containing both T- and B-cell growth factors (see below), were purified from peripheral blood lymphocyte-conditioned media by ammonium sulphate precipitation (50-75% precipitate), DEAE-Sepharose chromatography (0.07 M NaCl elution fraction) and Ultrogel filtration ( $\sim 25,000$ -molecular weight ( $M_r$ ) fraction). This material, at all stages of purification, was capable of stimulating <sup>3</sup>H-TdR incorporation in 7-day-old activated T cells and capable of maintaining long-term T-cell growth<sup>13</sup>.

Table 1 illustrates the ability of the soluble factors contained in interleukin preparations to stimulate B-cell <sup>3</sup>H-TdR incorporation in the presence of fetal calf serum (FCS). In experiment 1, B cells were purified from peripheral blood by negative selection; they were isolated from those cells remaining after a double adherence protocol and E-rosette depletion. These cells were ≥88% immunoglobulin-positive with the remaining cells composed of null cells, polymorphs and a small percentage of nonspecific esterase-positive cells. The B cells in experiment 2 were further purified by passage over a Sephadex G10 column<sup>14</sup>. These cells were >95% surface immunoglobulin-positive (SIg<sup>+</sup>) and contained less than 1% T cells as determined by reactivity to anti-Leu-1 monoclonal antibody. As expected, PWM stimulation of a quiescent B-cell population

Table 1 Effect of soluble factors in interleukin preparations on purified human B cells

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3H-TdR incorpo	oration (c.p.m.)
Expt 1	Expt 2
1,335	3,650
8,228	4,034
19,567	23,091
2.690	ND
40,176‡	38,931\$
	Expt 1 1,335 8,228 19,567 2,690

Cells were cultured in microtitre plates in a final volume of 0.2 ml. Total numbers of B cells added were  $0.2 \times 10^6$ , T cells (where added) were  $0.2 \times 10^6$  and MØ were  $0.04 \times 10^6$  (where added). The cells were grown for 48-72 h then labelled with 1 µCi 3H-TdR for the final 24 h. Standard errors of the mean were routinely <7%. In experiment 1, the B cells were prepared from peripheral venous blood by negative selection. E rosette-positive cells were removed on Ficoll gradients and the nonspecific esterase-positive cells removed by several adherence steps on Petri After these procedures the cell population was 88% SIg+ by immunofluorescence and contained <1% E rosette-positive cells. In experiment 2, peripheral blood B lymphocytes purified as described for experiment 1 were further processed by passage over a G10-Sephadex column. After this step, the recovered population contained 95% SIg+ cells and <1% T cells, as determined by E-rosetting and using anti-Leu-1 monoclonal antibody specific for human T cells. ND, not determined.

\* PWM was used at a final dilution of 10 µl per ml culture volume (Gibco). T cells were prepared from peripheral venous blood from the nonadherent. E rosette-positive, nylon wool-fractionated population as previously described21 The T cells were irradiated (1,500-2,000 rad) before co-cultivation with the B cells. After irradiation these T cells failed to incorporate significant <sup>3</sup>H-TdR in response to lectin exposure.

† In this experiment, IL-1 was used at a 1:100 final dilution. Further experi-

ments using multiple dilutions gave the same results.

‡ The IL-2 preparation (containing both B- and T-cell stimulatory factors) was processed by selective ammonium sulphate precipitation followed by DEAE-Sepharose chromatography, and was used at a final dilution of 1:10. To confirm that residual contaminating T cells (unexposed to lectin) in the B-cell population would not respond to DEAE-chromatographed conditioned medium alone (that is, the IL-2 preparation was free of residual lectin), we tested the preparation on quiescent peripheral blood lymphocytes in comparison with long-term cultured polyclonally activated T cells. <sup>3</sup>H-TdR incorporation of long-term cultured lines, in the presence of multiple dilutions of IL-2, was 16,924 ± 2,537 c.p.m. at 1:4 dilution and 214 ± 29 at 1:128 dilution. This is in striking contrast to peripheral blood lymphocytes, which showed 3H-TdR incorporation of only 73 and 20 c.p.m. at 1:4 and 1:128 dilution, respectively.

§ The IL-2 preparation (containing both B- and T-cell stimulatory factors) was

processed as in experiment 1 but was further fractionated by gel filtration on a Ultrogel AcA54 column. That activity eluting at Mr 24,000 was tested in experi-

ment 2, at a final dilution of 1:20.

Table 2 Evaluation of T-cell component in mitogenic assay of B-cell proliferation

	<sup>3</sup> H-TdR incorporation (c.p.m.)
B cells	2,967
B cells + PWM	7,186
Irradiated T cells	1,314
B cells + T cells (irradiated; $0.2 \times 10^6$ )*	20,302
+IL-2 (containing both B- and T-cell	
stimulatory factors)†	
B cells + T cells $(0.2 \times 10^6)$ ‡ + IL-2	22,066
B cells + T cells $(0.02 \times 10^6)$ + IL-2	19,353
B cells + T cells $(0.01 \times 10^6)$ + IL-2	21,309
B cells + T cells $(0.005 \times 10^6)$ + IL-2	21,314
B cells + T cells $(0.002 \times 10^6)$ + IL-2	20,223

B and T cells (where added) were incubated in 0.2 ml in microtitre wells for 48 h, then 1 μCi 3H-TdR was added to each well. After 24 h the cells were collected as described in Table 1 legend. The IL-2 preparations were added as 10% (v/v) of the final culture medium.

These T cells were irradiated (2,000 rad) before co-cultivation.

† The IL-2 preparation was fractionated through DEAE-Sepharose as described in Table 1. This IL-2 (and that in Tables 3 and 4) contains both T- and B-cell stimulatory factors. The IL-2 preparation was shown to stimulate <sup>3</sup>H-TdR incorporation of the magnitude demonstrated above in isolated B-cell preparations from multiple donors including the results shown in Table 1.

‡ These T cells and those in the following groups were not irradiated before co-cultivation.

was augmented in the presence of irradiated (2,000 rad) T cells and macrophage  $M\emptyset$ . Testing of the interleukins revealed that IL-1 had no activity when added by itself to B cells. However, the IL-2 preparations, fractionated by either DEAE-Sepharose chromatography or DEAE-Sepharose plus Ultrogel chromatography, contained moieties that stimulated B-cell populations; these preparations showed a stimulation index equal to or greater than that seen in the same cells after PWM stimulation. To ensure that any potential residual T-cell contamination of the purified B-cell population was not responsible for the DNA synthesis seen in Table 1, increasing numbers of nylon wool-purified T cells were added to the purified B cells and then stimulated with the soluble factors contained in the IL-2 preparations fractionated through DEAE-Sepharose chromatography (Table 2). As quiescent, non-lectin-activated T cells should not be stimulated by the lectin-free IL-2 preparations alone<sup>15,16</sup>, we expected that the T cells would not account for the increased thymidine incorporation observed. The results (Table 2) show that addition of irradiated or non-irradiated T cells did not increase the level of <sup>3</sup>H-TdR incorporation by B cells in response to the soluble factors in the IL-2 preparations.

As IL-2 has been shown to support T-cell blast proliferation in vitro, we next evaluated whether the IL-2 preparations would have any activity on PWM-stimulated B-cell blasts. We prepared B-cell blasts by adding PWM and irradiated syngeneic T cells to the B-cell population and incubating the cells in 5% CO<sub>2</sub> for 72-96 h. The irradiated T cells were then rosetted with sheep red blood cells (SRBC) and removed on Ficoll-Hypaque gradients while the  $M\emptyset$  component was removed by adherence. The B-cell blast population was then stimulated with the IL-2 preparations containing B-cell growth factor for 72 h and <sup>3</sup>H-TdR incorporation was measured during the last 24 h. Table 3 shows that soluble factors within DEAE-Sepharose-fractionated IL-2 preparations stimulated significant <sup>3</sup>H-TdR incorporation. Serial studies in which the blasts were re-stimulated with the B-cell growth factors at 72-h intervals, revealed that thymidine incorporation could be maintained for multiple reseedings and could be accounted for by B-cell stimulation alone. If the growth factor was deleted during these sequential studies, proliferation ceased. Furthermore, the percentage of

Table 3 Effect of soluble factors in interleukin preparations on PWM-stimulated human B-cell blasts

Days in culture	Cells labelled	<sup>3</sup> H-TdR incorporation (c.p.m.)	% Contamination by T cells
(	B cells	1,586	0
7 {	B cells B cells + IL-2 (containing both T- and B-cell stimulatory factors)*	7,824	1
	B cells	1,693	0
11 {	B cells B cells + IL-2	51,672	2
14 {	B cells B cells + IL-2	1,336	0
14	B cells + IL-2	28,256	0

\* Purified B cells were mixed with an equal number of purified, irradiated (1,500 rad) T cells and 10% MØ by concentration. The cells were stimulated for 96 h with PWM (10  $\mu$ l ml $^{-1}$ ) at which time the cells were depleted of adherent cells and E-rosettable cells as described in the text. The repurified B-cell blasts were then cultured in tubes (Falcon 3033) either in medium + FCS, or medium + FCS + the IL-2 preparation (1:10). The blasts were seeded at  $5\times10^5$  cells ml $^{-1}$  and re-fed with either medium–FCS alone or medium–FCS and the IL-2 preparations (containing both B- and T-cell stimulatory factors) every 72 h in association with re-feeding at the original concentration. In all cases, the B blasts were re-fed every 72 h; 24 h before re-feeding, an aliquot was labelled with 1  $\mu$ Ci  $^3$ H-TdR and the acid-precipitable material collected on Whatman GF/C filters and counted. Results represent the mean c.p.m. per assay point for a single experiment, with replicate experiments indicating the same trend; s.e.m. of cell population, <15%.

 Table 4
 Effect of soluble factors in Ultrogel-fractionated interleukin-2 preparations on B cells

Dilution of	<sup>3</sup> H-TdR incorporation (c.p.m.)*								
IL-2	Quiescent B cells	B-cell blasts†							
attackers.	$705 \pm 86$	$2,438 \pm 421$							
0.1%	$606 \pm 53$	$3,116 \pm 1,001$							
0.5%	$3,047 \pm 943$	$16,067 \pm 854$							
1.0%	$3,340 \pm 418$	$7,455 \pm 2,983$							
5.0%	$16,670 \pm 4,868$	$18,465 \pm 1,402$							
10.0%	$9,639 \pm 3,326$	$18,602 \pm 862$							

The IL-2 preparation was purified by DEAE-Sepharose and Ultrogel chromatography. Active material eluting from the DEAE-Sepharose column was stabilized with polyethylene glycol  $^{13}$ . The active fraction was then placed on an Ultrogel AcA54 column and the peak of T-cell activity eluting in the  $\sim\!24,000\text{-}M_r$  range collected. This material (0.1% final volume) acting on lectin-stimulated T-cell blasts in a microtitre assay, gave 1,393 c.p.m.  $^3\text{H-TdR}$  incorporation at 72 h, whereas a 10.0% final volume gave 18,323 c.p.m. incorporated.

\* Cells were placed in microtitre wells as described in Table 1 legend except that, 24 h before collection, they were labelled with  $0.5~\mu Ci^3$ H-TdR.

† B-cell blasts were prepared by stimulating purified B cells for 24 h in the presence of PWM. At the time of the microtitre assay, the PWM was washed from the cells and the IL-2 preparation added as above.

E-rosettable cells did not increase with time (Table 3), which strongly argues against the possibility that either T cells or null cells developing T markers account for the proliferation observed.

The results in Table 4, together with the data of Table 1, experiment 2, further demonstrate the presence of B-cell stimulatory factors in those IL-2 preparations that have been fractionated further by Ultrogel chromatography<sup>13</sup>. This material has been shown to contain a minimum of four bands discernible by gel electrophoresis. The factor(s) present in this fraction were again able to support the incorporation of <sup>3</sup>H-TdR by purified B cells as well as purified T cells.

These data reinforce the concept that normal human B lymphocytes are auxotrophic, requiring accessory cell signals for activation<sup>17,18</sup>. Most human B-cell mitogens such as PWM and Staphylococcus Protein A have been shown to be predominantly T-cell dependent in man<sup>19,20</sup>. Various T-cell helper factors such as AEF<sup>21</sup>, LMF<sup>22</sup>, and T-cell replacing factor<sup>23</sup>, all require T cells for their production. Reinherz et al.<sup>24</sup> have shown that LMF is a product of a monoclonal antibody-defined T helper subset. Macrophages are also apparently involved in B-cell activation by facilitating the interaction with helper T cells<sup>25</sup>, and later by stimulating differentiation<sup>26</sup>. Our study suggests that the interleukins as presently purified belong to this family of T-cell helper factors and factors within the preparations will stimulate <sup>3</sup>H-TdR incorporation in both quiescent B cells as well as activated B-cell blasts in FCS.

The major question now is whether this proliferative response of B cells to the soluble factors in the interleukin 2 preparations is due to a nonspecific mitogenic effect of the putative T-cellspecific lymphokine or due to a co-purifying B-cell-specific growth factor. Experiments are being done to resolve this question by means of differential factor absorption from the IL-2 preparations. Initial observations indicate that when the IL-2 preparations are absorbed by a population of B cells and then mitogenically tested on lectin-activated T cells, the activity of the absorbed material is frequently more active than a preparation that has not been absorbed. The converse experiment, where the IL-2 preparations are absorbed by T cells and then tested on B cells, also reveals a frequent augmentation of the B-cell response compared with material that has not been absorbed (data not shown). Furthermore, absorption of the IL-2 preparations by B cells fails to remove T-cell-specific activity and T-cell absorption fails to remove the activity that acts on B cells. These preliminary studies suggest that either absorption leads to factor release in the media, or that the interleukin preparations contain co-purifying but separate B- and T-cellspecific stimulatory factors.

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## Tumour promoters induce mitotic aneuploidy in yeast

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Tumour promoters, which complete the process of carcinogenesis initiated by subthreshold doses of carcinogens, can effect changes in gene regulation and cellular differentiation<sup>1</sup> Whether tumour promoters also induce genotypic changes is unclear. 12-O-tetradecanoylphorbol-13-acetate (TPA), potent tumour promoter, is inactive in gene mutation assays<sup>1-6</sup>, but has been reported by some<sup>6,7</sup>, though not others<sup>3-1,9</sup>, to induce sister chromatid exchanges, which may be cytological indications of recombinational events<sup>11</sup>, in mammalian cells. However, TPA alone is not recombinogenic in yeart<sup>12</sup>. We now report that in a genetic system using the yeast Saccharomyces cerevisiae 13-18, TPA fails to induce recombination but induces mitotic aneuploidy—a change in chromosome number which may lead to hembygodity of recessive mutations or to phenotypic changes due to an alteration in gene balance. The ability of other agents to induce aneuploidy in this system also correlates with their tumour-promoting activity.

The compounds studied were assayed for their abilities to induce mitotic an euploidy (see Fig. 1 for method) and crossing-over in the  $D_6$  strain of S. cerevislae <sup>13-15</sup>, a diploid strain that carries a series of recessive, coupled markers on chromosome VII. The compounds tested included several promoters and structurally related nonpromoters. All promoters tested for the ability to induce aneuploidy were positive, whereas nonpromoters were negative (Table 1). Figure 2 plots the dose-response curve for TPA induction of aneuploidy, showing that dose-related increases in mitotic aneuploidy were obtained over the concentration range 0.02-5 μg ml<sup>-1</sup>. Phorbol-12-13didecanoate (PDD), another potent promoter in the phorbol ester series16, was also positive (Table 1). In contrast, no significant increases in aneuploidy were observed with phorbol, phorbol monoesters, 4-O-methyl-TPA or  $4\alpha$ -PDD, which are also inactive as promoters on mouse skin<sup>16</sup>

Of particular interest is mezerein—a weak promoter, but as potent as TPA in inducing many biochemical and morphological effects in cells 15,17. In a two-stage promotion protocol on mouse skin<sup>17</sup>, mezerein is an incomplete promoter, that is, it is not effective in the first stage of promotion but is as effective as TPA in the second stage. In the concentration range 0.01-100 µg ml<sup>-1</sup>, mezerein did not induce mitotic aneuploidy in our RESERV

Other structurally unrelated promoters, including anthralin18, lodoacetic acid19, saccharin20, oleic acid21,22 and lauric acid22 did induce mitotic aneuploidy. Stearic acid, a nonpromoter22, was inactive. Diethylstilboestrol, which may be a tumour promoter as well as initiator23, was positive.

In contrast with the ability of the tumour promoters to induce mitotic aneuploidy, they had no effect on the frequencies of mitotic crossing-over. However, Fig. 3 shows that, in identical conditions, the incomplete promoter mezerein did induce mitotic crossing-over at concentrations >10 µg ml<sup>-1</sup> whereas TPA was ineffective. Mezerein was the only compound tested which showed significant activity in the crossing-over assays.

The expression of recessive mutations resulting from homozygosity produced by mitotic recombination has been suggested as an important step in tumour promotion. However, with the exception of the incomplete promoter mezerein, none of the compounds tested was capable of inducing detectable : levels of mitotic crossing-over in the yeast strain used here. In view of the relative ease with which mitotic crossing-over may be detected in yeast after treatment with complete carcinogens and initiating agents<sup>24</sup>, it seems unlikely that the tumour promoters tested are capable of producing significant levels of mitotic recombination. The potential significance of the ability of mezerein to induce mitotic crossing-over is not clear.

Boutwell<sup>25</sup> has proposed that the promotion phase of carcinogenesis consists of at least two parts: phase I involving 'conversion' of the initiated cell and phase II the 'propagation' of the converted, initiated cell<sup>25</sup>. Complete promoters, such as TPA, can induce both phases but some substances, such as mezerein, are incomplete promoters and can induce only the second phase<sup>17</sup>. Recently, Slaga and co-workers<sup>17</sup> confirmed this multi-stage model of tumour promotion and demonstrated that the conversion phase, like the initiation phase of carcinogenesis, requires only a single exposure to the cocarcinogen, although a dose-related increase occurs with additional exposures<sup>17</sup>. Furthermore, the conversion phase is irreversible, for at least 4 weeks after a single exposure to TPA (T. Slaga, personal communication). It is possible that the conversion phase of promotion requires a genetic change induced by the tumour promoter. The correlation of aneuploidy induction with promoters, nonpromoters and the incomplete promoter mezerein is consistent with a role for chromosome changes in this phase. The epigenetic changes induced by promoters in gene regulation and cellular differentiation are probably also important in overall tumour promotion.

We have shown an apparent correlation between the ability of a compound to induce mitotic aneuploidy in S. cerevisiae and its ability to act as a tumour promoter. While such a correlation may be coincidental, we suggest that it reflects a mechanistic relationship between chromosome aneuploidy and tumour promotion. Most chemical carcinogens have been shown to be capable of inducing point mutations in experimental systems? however no such activity has been demonstrated for tumour

Table 1 Correlation of tumour-promoting activity and induction of aneuploidy or crossing-over in assays using yeast strain D<sub>6</sub>

Compound Phorbol analogues	Tumour promoting	Response in aneuploidy (conc. range in µg ml <sup>-1</sup> )	Monosomic colonies per 10 <sup>6</sup> viable cells Control Treated (in µg ml <sup>-1</sup> )	No. observed colonies/ no. plates	Response in mitotic crossing-over assay (conc. in µg ml <sup>-1</sup> )	Red, cycloheximide- resistant, requiring adenine & methionine per 10 <sup>4</sup> viable cells Control Treated (in µg ml <sup>-1</sup> )	No. observed colonies/ no. plates
1. TPA	Positive	Positive	$0-3.7\pm0.4$	32/4	Negative	$0-4.7\pm0.8$	41/4
		(0.02-5)	$0.5-41.5\pm6.2$	356/4	(up to 100)	$20-4.5\pm1.2$	40/4
2. Phorbol-12,13-	Positive	Positive	$0-4.2\pm0.7$	41/4	Negative	$0-7.0\pm1.0$	69/4
didecanoate (PDD)		(1-10)	$3-52.2 \pm 9.0$	353/4	(up to 50)	$10-8.8\pm1.5$	71/4
3. Phorbol	Negative	Negative	$0-4.2\pm0.7$	41/4	Negative	$0-7.0\pm1.0$	69/4
		(up to 50)	$10-5.2\pm1.2$	52/4	(up to 50)	$20-8.3\pm1.8$	67/4
<ol> <li>4. 12-O-tetradecanoyl-</li> </ol>	Negative	Negative	$0-4.7\pm1.4$	44/4	Negative	$0-9.3\pm1.3$	87/4
phorbol		(up to 50)	$20-4.9\pm1.2$	45/4	(up to 50)	$30-7.3\pm1.1$	58/4
<ol><li>Phorbol-13-acetate</li></ol>	Negative	Negative	$0-4.4\pm1.6$	37/4	Negative	$0-17.6 \pm 2.5$	147/4
		(up to 50)	$20-9.1\pm2.2$	79/4	(up to 50)	$10-24.2 \pm 2.9$	172/4
6. 4-O-methyl-TPA	Negative	Negative	$0-4.4\pm1.6$	37/4	Negative	$0-17.6 \pm 2.5$	147/4
		(up to 50)	$30-5.1\pm1.9$	22/4	(up to 50)	$30-19.1\pm2.8$	81/4
7. 4α-PDD	Negative	Negative	$0-4.2\pm0.7$	41/4	Negative	$0-7.0\pm1.0$	69/4
		(up to 50)	$20  6.1 \pm 0.8$	52/4	(up to 50)	$40-8.0\pm1.1$	51/4
Structurally unrelated con	nnounds						
8. Anthralin	Positive	Positive	$0-4.4\pm1.6$	37/4	Negative	$0-17.6 \pm 2.5$	147/4
		(0.5-5)	$1.5 - 28.7 \pm 4.4$	162/4	(up to 50)	$40-24.5\pm5.6$	54/4
9. Iodoacetic acid	Positive	Positive	$0-4.2\pm0.7$	41/4	Negative	$0-7.0\pm1.0$	69/4
×	·	(5-50)	$20-21.8\pm3.3$	121/4	(up to 100)	$40-11.7\pm1.2$	40/4
10. Oleic acid	Positive	Positive	$0-4.2\pm0.7$	41/4	Negative	$0-7.0\pm1.0$	69/4
ro. Giele dela	1 Ostive	(100-500)	$300-16.3\pm2.1$	116/4	(up to 50)	$50-11.1\pm1.5$	92/4
11. Lauric acid	Positive	Positive	$0-9.1\pm1.2$	89/4	Negative	$0-12.7\pm1.3$	111/4
11. Euglie ucid	1 Osttive	(10-200)	$200-29.7\pm3.2$	191/4	(up to 50)	$50-13.1\pm1.8$	79/4
12. Stearic acid	Negative	Negative	$0-9.1\pm1.2$	89/4	Negative	$0-12.7\pm1.3$	111/4
is ordanic acid	· · · · · · · · · · · · · · · · · · ·	(up to 500)	$400-15.5\pm2.4$	130/4	(up to 500)	$300-15.8\pm3.4$	47/4
13. Diethylstilboestrol	?	Positive Positive	$0-8.1\pm2.0$	91/4	Negative	$0-4.5\pm0.6$	51/4
15. Diemyiamodestroi	*	(20-200)	$50-59.8\pm10.2$	134/4	(up to 200)	$25-7.4\pm1.9$	27/4
14. Saccharin	Positive	Positive	$0-10.6\pm1.2$	110/4	Negative	$0-7.5\pm1.3$	78/4
i ii Gueerailli	1 0311110	(200-400)	$350-153\pm23.3$	410/4	(up to 500)	$200-9.4\pm2.0$	47/4
15. Mezerein	Incomplete	Negative	$0-3.7\pm0.4$	32/4	Positive	$0-4.7\pm0.8$	41/4
io, meetion	incomplete	(up to 100)	$3-4.7\pm0.8$	36/4	(20–100)	$30-62.2\pm9.2$	122/4
		(up to 100)	J= 4.7 ± 0.6	30/4	(20-100)	JU- 02.2 x 9.2	144/4

Concentrations are those tested and, in most cases, represent those over which cell viability of tested cells was > 10% of the control value. At high levels of cell lethality, the reliability of the assay was reduced. A positive response was a threefold or greater increase in the frequency of aneuploid colonies in at least two separate experiments. In the case of phorbol-13-acetate, a small (twofold) increase was observed but the results obtained were not significantly different from the control values. A negative response was indicated if no significant increase in the frequency of aneuploid colonies or mitotic crossing-over occurred in either the left or right arm of chromosome VII. In the case of compounds 1, 2, 3, 4, 5, 6, 7, 9 and 13, no increases have been observed in the frequencies of induced mitotic recombination (including both gene conversion and crossing-over) in a variety of strains of yeast. The table also illustrates the maximum frequency of both induced aneuploidy and crossing-over observed in a single experiment. All the experiments used yeast cultures alone without the presence of an extracellular activating system involving rat liver microsomes (S9 mix). In the case of 12-O-tetradecanoyl-13-acetate, S9 mix reduced the induced frequency of aneuploid cells. The level of aneuploid cells in the control cultures was  $3-9 \times 10^{-6}$  viable cells. Compounds were from: 1-6, Dr Peter Borchert (Chemical Carcinogenesis, Minnesota); 7, Consolidated Midland (New York); 9-13, Sigma; compound 8 was a gift from Dr Tom Slaga (Oak Ridge National Laboratory).

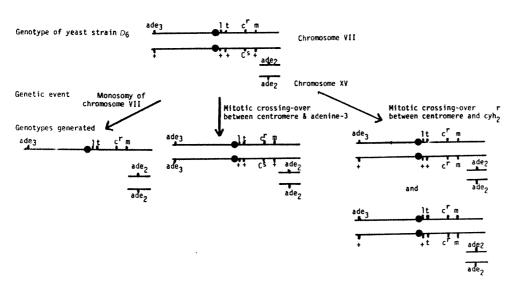


Fig. 1 Principles involved in the detection of mitotic aneuploidy in strain D6. Cells homozygous for the ade2 mutation require adenine for growth and produce red colonies; homozygosity of the ade3 mutation, which occurs at an earlier step in adenine synthesis, leads to the production of white, adenine-requiring colonies. Homozygosity of all the markers on chromosome VII may also result from simultaneous crossing-over in both the left and right arm of chromosome VII; however, such events are measurable by the frequency of crossing-over assayed in both arms separately. The possible role of such multiple cross-over types in the origin of white, cycloheximide-resistant colonies has been extensively discussed elsewhere 13. l, Leucine; t, tryptophan; c', cycloheximide resistance-2; methionine 13, C\* = cycloheximide sensitivity-2.

Phenotypes

white, cycloheximide resistant, requiring adenine, leucine, tryptophan and methionine

white, cycloheximide sensitive, requiring adenine

red, cycloheximide resistant, requiring adenine and methionine

promoters, which seem incapable of direct interaction with DNA. Our data support the hypothesis that tumour promoters act by stimulating the expression of recessive mutations (presumably in regulatory genes) produced by an initiating agent or by altering gene balance, both of which could result from changes in chromosome number as a result of chromosome aneuploidy. Such changes in chromosome number have frequently been observed in the karyotypes of tumour cells27 and the data reported here suggest they are causal in tumour

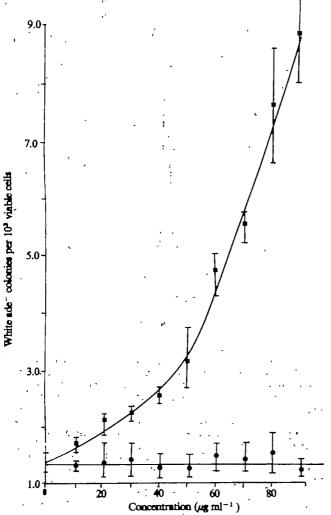


Fig. 2 Induction of mitotic anemploidy in cultures of yeast strain D<sub>4</sub> after exposure to TPA ( $\bullet$ ) or mezerein ( $\blacksquare$ ). Cultures of strain D<sub>4</sub> were collected during exponential growth, washed and resuspended in pH 7.0 phosphate buffer at a cell concentration of  $10^7$  ml $^{-1}$ . Cell samples (2 ml) was incubated at 30 °C in scaled bottles in a shaking water bath for 6 h in the presence of TPA or mezerein at concentrations of up to 5µg ml<sup>-1</sup> (both compounds were prepared as stock solutions in dimethyl sulphoxide). Complete growth medium (2 ml) was then added to each bottle, and staking and incubation continued for a further 18 h. In the experiment shown, 18 ml of growth medium were added. Cultures were washed three times by centrifugation, resuspended in sterile saline and plated after suitable dilution on year complete medium to score cell viability and on yeast complete medium plus of cycloheximide to score white, cycloheximide-resi monosomic (2n-1) colonies. All treated cultures were examined microscopically after washing and in no case was there evidence of sporulation amongst the diploid cells. Viability plates were scored after 5 days and the frequency of monosomic colonies after-10 days of incubation at 28 °C. Representative samples of white, cycloheximide-resistant colonies were replicated onto selective minimal plates to determine the expression of the recessive markers on chromosome VII of strain De. All compounds reported here as inducing mitotic aneuploidy gave rise to white, cycloheximide-resistant colonies requiring adenine, leucine, tryptophan and methionine, as would be expected of cells which have lost one copy of chromosome VII. Details of the genetic basis of this assay are described in ref. 14. It is technically possible that some homozygous colonies arose from melotic recombination during the treatment period; however, in none of the cultures sed recombination would have been moiosis observed and incres obsérvable in our crossing-over assay.

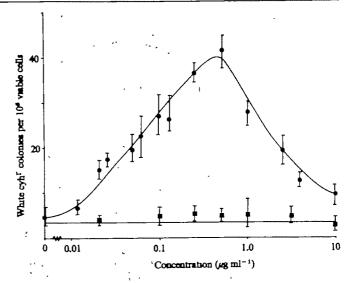


Fig. 3 Induction of mitotic crossing-over in cultures of yeast strain D<sub>6</sub> after exposure to TPA (\*\*) and mezerein (\*\*\*). Cultures were exposed to either compound at concentrations of up to 100 µg ml<sup>-1</sup> in the conditions described in Fig. 2 legend. After treatment, cultures were plated for cell viability, for the induction of white, cycloheximide-registrant, monoscomic colonies and for the frequency of white, cycloheximide-sensitive colonies produced on yeast complete growth medium at dilutions giving 10 times more viable cells than on the viability plates. All treated cultures were examined microscopically after washing and in no case was there any evidence of sporulation among the diploid cells. White, cyclohexumdesensitive colonies were replicated onto supplemented (with leucine, tryptophan and methionine) minimal plates. More than 90% of the white colonies are adenine requiring and result from mitotic crossing-over between the centromere and the edestre-3 gene on the left arm of chromosome VII. Assays of mitotic crossing-over were also performed using the cyclohextmide-2 gene on the right arm of chromosome VII with similar results.

development. The changes in chromosome aneuploidy would readily fit the multi-stage models of carcinogenesis.

The data presented here do not indicate whether the assay of induced chromosome aneuploidy in yeast will be useful in detecting tumour promoting chemicals. Such a conclusion awaits the demonstration that chromosome aneuploidy is also induced in mammalian cells by tumour promoters.

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# Isolation of I-A subregion-like molecules from subhuman primates and man

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Immune response-associated (Ia) molecules play a major part in cellular interactions that initiate an immune response. Two families of Ia molecules, determined by the I-A and I-E subregions of the murine major histocompatibility complex (MHC), have been isolated and characterized in mice1. I-A and I-E molecules each consist of two polypeptide chains (molecular weights  $(M_rs)$  of 26,000 and 35,000 for I-A and 24,000 and 32,000 for I-E) that are noncovalently associated2. Their large subunits are designated  $A_{\alpha}$  and  $E_{\alpha}$ , respectively, and their small subunits  $A_{\beta}$  and  $E_{\beta}$ . Despite the similarity in their subunit composition, the I-A and I-E molecules are structurally distinct according to peptide mapping and partial N-terminal sequence analysis<sup>3,4</sup>. Although the structural homologue of murine I-E molecules has been identified in man and is known as HLA-DR5, the I-A equivalent has never been described. In an attempt to identify the human analogue of the murine I-A molecule, murine monoclonal antibodies were produced after immunization with a mixture of three putative human cell lines. We report here that one of these antibodies reacts with I-A-like molecules from two cell lines, GM3158, which after karyotype analysis was shown to have originated not from man but from a marmoset, and GM3163, a cell line of human origin.

Monoclonal antibodies to cell surface molecules were produced by immunizing BALB/c mice with a mixture of the cell lines GM3107, GM3158 and GM3163 (obtained from the Human Mutant Cell Repository, Camden, New Jersey) and fusing the immune splenocytes with SP2/O-Ag cells. After appropriate screening by indirect immunoprecipitation, several positive clones were identified—two of these, designated SG157 and SG171, were analysed further. When tested with a lentil lectin-purified glycoprotein fraction from cell line GM3158, the monoclonal antibodies precipitated molecules having two, slightly different profiles after SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 1). SG157 precipitated products with SDS-PAGE profiles characteristic of human HLA-DR antigens: two polypeptides of Mrs ~29,000 and 34,000, respectively with the smaller subunit  $(\beta)$  displaying the characteristic retardation in migration rate in reducing conditions<sup>2</sup>. In contrast, SG171 precipitated two molecules of different molecular weights (~30,000 and 33,000); in reducing conditions these merge into a single peak with an apparent  $M_r$  of 33,000. Peptide map comparisons (Fig. 2) of the molecules isolated with SG157 and SG171 reveal striking differences which suggest that, despite similarities in their molecular weights, the molecules detected by SG157 and SG171 are unrelated.

The molecules binding to SG157 and SG171 were identified by limited amino acid sequence analysis (Table 1). The two polypeptide chains reacting with SG157 show a high degree of sequence identity, 9 of 11 residues (82%), with the two polypeptide chains of the murine I–E molecule and thus represent the marmoset analogue. In contrast, the two polypeptide chains reacting with SG171 are homologous to the murine I–A molecule. The large ( $\alpha$ ) subunit of the molecule binding to SG171 is identical to a prototype of the large subunit of murine I–A molecules ( $A_{\alpha}$ ) in 5 of 8 sequence residues that can be compared, provided a gap is inserted at the amino terminus.

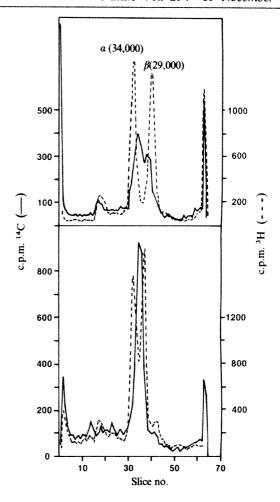


Fig. 1 SDS-PAGE profiles of molecules isolated from cell line GM3158 with SG157 (---) and SG171 (- monoclonal antibodies in non-reducing (top) and reducing (bottom) conditions. Cells  $(2 \times 10^8)$  were labelled in culture with either <sup>3</sup>H- or phenylalanine and their glycoprotein components were isolated as described elsewhere8. The relevant molecules were isolated by adsorption of the radiolabelled glycoprotein fractions on to an immunoadsorbent column consisting of monoclonal antibody (SG157 or SG171) covalently coupled to Sepharose-4B, followed by elution with 0.05 M diethylamine. The eluted material was lyophilized, dissolved in water and acetone precipitated. The precipitates were dissolved in Laemmli<sup>14</sup> non-reducing sample buffer and <sup>14</sup>C- and <sup>3</sup>H-labelled material was mixed in a 2:1 ratio. One half of the sample was reduced with 2-mercaptoethanol. The samples were electrophoresed on 12.5% polyacrylamide gels and the gels were sliced into 1 mm thick sections. After overnight incubation in water the entire slice, as well as the eluted material, was counted in a liquid scintillation counter. Radioactivity (c.p.m.) was corrected for <sup>3</sup>H and <sup>14</sup>C channel spillover.

Furthermore, the small subunit  $(\beta)$  is identical to that of the murine I-A molecule  $(A_{\beta})$  in 6 of 7 sequence residues that can be compared. The combined degree of homology, 11 of 15 residues (73%), is comparable with that observed between other MHC products of different species (that is, mouse and man)<sup>5,6</sup>, and strongly suggests that the molecule reacting with SG171 is the homologue of the murine I-A molecule.

The marmoset I-A molecule differs in several respects from its murine counterpart. Although both consist of two noncovalently associated subunits of similar molecular weight, the two subunits of the murine I-A molecule are generally better resolved on SDS-polyacrylamide gels<sup>2</sup>. In addition, whereas reducing conditions do not appreciably alter the migration rate of either subunit of murine I-A molecules compared with those of the unreduced forms when analysed by SDS-PAGE, the small

	T	able 1	Parti	al NH <sub>2</sub> -	termin	al seque	ences o	f I–A- a	nd I-E	(DR)-l	ike mo	lecules i	solated	from ce	ell line	GM31	58	ny taona amin'ny taona amin'ny faritr'i Amerika.	
									Residu	e									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Homology
(SG157) α Murine I–E α	Ile Ile	Lys			His	Thr	Ile Ile	Ile Ile		Ala		Phe Phe	Tyr Tyr	Leu Leu	Val Leu	Pro			6/7
(SG157) β Murine I–E β	[] Val	Arg		Ser	Arg	Pro		Phe Phe	Leu Leu		Tyr	Leu Ser Val	Lys Thr	Ser				Phe Phe	3/4
(SG171) α Murine I–A α	[]			Ile Ile	Val —	Ala Ala	,		Val Val		Val Phe	Tyr Tyr		Val —		Val	Tyr Tyr		5/8
(SG171) β Murine I-A β			Ser		Arg	His	Phe Phe	Val Val	Tyr Val Phe		Phe Phe	Pro Ser	Pro Lys	Phe , Phe		Tyr Tyr			6/7

Cells were radiolabelled in vitro with several <sup>3</sup>H-amino acids and SG157 and SG171 molecules were isolated and purified as described in Fig. 1 legend. Proteins were sequenced and residues identified as described elsewhere. Boxed residues represent identities between species and dashes indicate presence of residue found in one species but absent from the other. The murine sequence data were compiled from refs 3, 5, 12 and 13.

subunits of marmoset I-A- and I-E-like molecules migrate more rapidly in non-reducing conditions. Furthermore, one must insert a gap at the N-terminus of the large subunit of the marmoset I-A-like molecule to align it with the large subunit of the murine I-A molecule. A similar requirement applies to the I-E(DR)-like molecule, for which a gap must be inserted at the N-terminus of the small subunit to align it with the small subunit of the murine I-E molecule<sup>7</sup>. The significance, if any, of these similarities between I-A and I-E-like molecules in marmosets is unknown.

The recognition that GM3158 is a marmoset cell line explains our previous observation that the  $DR_{\alpha}$  chain isolated from GM3158 differs structurally from the  $DR_{\alpha}$  chains isolated from two human cell lines, GM3107 and GM3163 (ref. 8). Although the  $DR_{\alpha}$  subunit is essentially invariant in structure within a species (mouse or man)<sup>9-11</sup>, its structure obviously varies between species.

Identification of an I-A-like molecule in subhuman primates strongly suggests that this molecule also exists in man. Indeed,

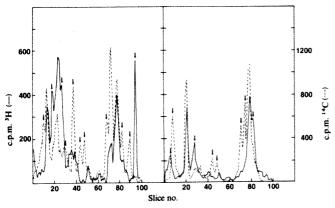


Fig. 2 Tryptic peptide comparisons of  $\alpha$  (left) and  $\beta$  (right) polypeptides isolated from GM3158 with monoclonal antibodies SG157 (---) and SG171 (----).  $^3$ H- and  $^{14}$ C-phenylalanine-labelled molecules were isolated with SG171 and SG157, respectively, and the component  $\alpha$  and  $\beta$  subunits separated by SDS-PAGE as described in Fig. 1 legend. Equivalent c.p.m. of  $^{14}$ C ( $\alpha$  or  $\beta$ )- and  $^3$ H ( $\alpha$  or  $\beta$ )-polypeptides were combined. After complete reduction, alkylation and trypsin-TPCK (tosyl-phenylethyl-chloromethyl-ketone) digestion, the peptides were separated on isoelectrofocusing gels as described elsewhere  $^8$ . The gels were sliced into 1-mm sections, incubated in water overnight and measured for radioactivity, which was plotted against slice number after correcting for  $^3$ H and  $^{14}$ C channel spillover. Arrows denote peptide differences.

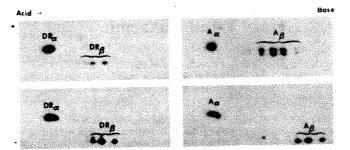


Fig. 3 Two-dimensional gel electrophoresis of DR- and I-A-like molecules isolated from cell lines GM3158 and GM3163. The DR- and I-A-like molecules were purified by immunoadsorption as described in Fig. 1 legend, except that Triton X-100 instead of deoxycholate was used in the elution step. The glycoprotein extract from GM3163 was first depleted of SG157-reactive material before being combined with the SG171 immunoadsorbent. The isolated material was analysed as described elsewhere  $^{15}$  except that non-reducing conditions were used throughout. The DR- and I-A-like molecules isolated from marmoset (top row) and man (bottom row) are distinguishable primarily by the difference in isoelectric points of the DR $_{\beta}$  and A $_{\beta}$  polypeptides. The marmoset and human I-A-like molecules have  $\sim\!90\%$  amino acid sequence homology (to be published).

preliminary results indicate that SG171 also reacts with an I-A-like molecule from some human cell lines. Two-dimensional gel analysis of molecules isolated from cell line GM3163 (confirmed by karyotype analysis to be of human origin) with SG157 and SG171 revealed DR- and I-A-like molecules respectively (Fig. 3). As in the marmoset, the  $A_{\beta}$  polypeptide is more basic than the DR\_{\beta} polypeptide. However, in contrast to the marmoset, human DR- and I-A-like molecules have almost identical molecular weights. It is also intriguing to note that the  $A_{\alpha}$  and DR\_{\alpha} subunits have not only identical molecular weights but also isoelectric points, suggesting that despite a lack of similarity in their NH\_2-terminal amino acid sequences, they may be evolutionarily related.

The similarity in molecular weights of the DR- and I-A-like molecules in man may explain why others have failed to identify the latter. It is likely that I-A-like molecules have been isolated but mistakenly identified as DR because of their similar migration rates when analysed by SDS-PAGE.

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# Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sézary T-cell leukaemia

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Retroviruses have been isolated from many animal species and several have been shown to be the aetiological agents of naturally occurring leukaemias, lymphomas and sarcomas (for recent reviews see ref. 1). There is evidence that these viruses or components of them are present in humans, but the isolation and analysis of retroviruses from human cells and evidence for an association with disease have been difficult to demonstrate (see refs 2, 3). We now report the isolation and characterization of a new type C retrovirus (HTLV<sub>MB</sub>) from a patient with a cutaneous T-cell leukaemia (Sézary syndrome). Analysis of the proteins and nucleic acids of this retrovirus isolate indicates that it is closely related to HTLV<sub>CR</sub>, a retrovirus recently isolated and characterized<sup>5-7</sup> from a patient with cutaneous T-cell lymphoma (mycosis fungoides) but distinct from known animal retroviruses. Proteins and nucleic acids of HTLV were also identified in the fresh (uncultured) Sézary leukaemic blood cells from which the second isolate was derived.

Retrovirus particles are usually not demonstrable until cells are successfully grown in culture. As T cells are often target cells for transformation by type C retroviruses, it is necessary to grow human T cells in continuous culture to determine whether they contain these viruses. The discovery of T-cell growth factor (TCGF)<sup>8,9</sup> and its recent purification from human lymphocyte conditioned media 10 have now made it possible routinely to grow normal T cells for long periods if the TCGF is added periodically to lectin- or antigen-activated lymphocytes. Neoplastic cells can be separated and grown in culture without normal T cells by using purified TCGF because many neoplastic T cells differ from normal T cells in not requiring in vitro activation by lectin to interact with TCGF<sup>11</sup>. Some cultured neoplastic T cells become independent of exogenous TCGF<sup>11,12</sup> probably because they become constitutive TCGF producers<sup>13</sup>. The use of TCGF has thus enabled neoplastic cell lines to be established and their retrovirus-producing properties analysed.

Mycosis fungoides and Sézary syndrome are two distinct clinical variants of a group of neoplastic diseases referred to as

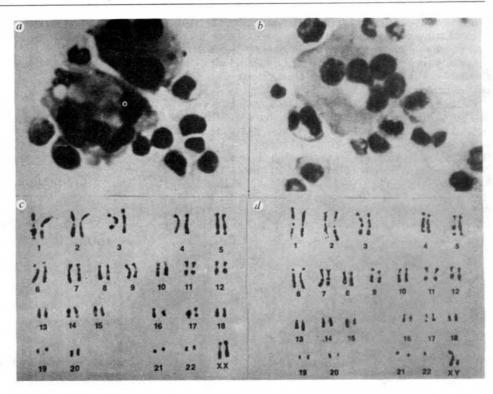
cutaneous T-cell lymphomas and leukaemias respectively14. The detailed clinical history of patient C.R. with mycosis fungoides has been published elsewhere<sup>4</sup>. The cell line, HUT102, was established from neoplastic T cells derived from his right inguinal lymph node<sup>12</sup>, using crude TCGF<sup>8</sup>. One year later, the cell line CTCL-3 was established from his peripheral blood using a lectin-free partially purified TCGF<sup>11</sup>. Patient M.B. was a 64-yr old black female with the leukaemic phase of Sézary syndrome. Examination of her peripheral blood revealed a white blood cell count of 300,000 mm<sup>-3</sup> with a differential of 72% lymphocytes, 35% of which had markedly convoluted nuclei, characteristic of Sézary leukaemic cells. The cell line CTCL-2 was established<sup>11</sup>, again using partially purified TCGF<sup>10</sup>, from a sample of her peripheral blood.

The characteristics of the three cell lines are similar and have been published in detail previously<sup>11,12</sup>. HUT102 and CTCL-2 are now grown independently of exogenous TCGF, while CTCL-3 remains dependent on its addition. Their morphology is that of atypical mono- and multi-nucleated lymphoblasts (Fig. 1a,b). Typical of T lymphoblasts, they form rosettes with sheep erythrocytes and are negative for Epstein-Barr virus nuclear antigen. Their histochemical staining pattern (negative for myeloid stains but positive for  $\alpha$ -naphthyl acetate esterase and acid phosphatase) and lack of terminal transferase activity are compatible with published reports on fresh cells from their disease of origin. Karyotypes performed on the cultured cells (Fig. 1c,d) are the same as those performed on the original fresh tissue source and have remained constant throughout culture (2 yr).

HUT102 and CTCL-3 are now constitutive producers of type C retrovirus particles (HTLV<sub>CR</sub>), while fresh peripheral blood lymphocytes from patient C.R. produced particles only after induction with 5-iodo-2'-deoxyuridine (IUdR). Similarly, CTCL-2 cells have produced typical type C particles (Fig. 2) from the earliest passage studied (passage 2), but only after induction with IUdR. Figure 3 summarizes the isolations of these viruses. As the cell lines are relatively poor producers of virus, large amounts of cells must be cultured for analytical studies. Typically, 20-501 of cells (10<sup>6</sup> per ml) are grown in RPMI-1640 with 10% fetal calf serum, and in the case of CTCL-2, the cells are also treated with IUdR (20 µg ml<sup>-1</sup>) for 24 h. The virus-releasing cells are then centrifuged, resuspended in fresh media and the media collected 72 h later. Virus particles are concentrated and purified by sequential banding in sucrose, pelleting through 30% glycerol and finally isopycnic banding in a continuous sucrose gradient. The fractions containing HTLV<sub>MB</sub> particles are identified by electron microscopy and by assaying for viral reverse transcriptase. These methods have been previously described for HTLV<sub>CR</sub> (ref. 4). As with  $\rm HTLV_{CR}$  and most other retroviruses, concentrated  $\rm HTLV_{MB}$  particles band at a density of 1.16 g ml<sup>-1</sup> and are associated with an endogenous DNA polymerase activity. The polymerase shows a preference for the synthetic template primers  $poly(A) \cdot oligo(dT) \quad and \quad poly(C) \cdot oligo(dG) \quad over \quad poly(dA) \cdot$ oligo(dT), characteristic of viral reverse transcriptase<sup>15</sup>. Like  $\rm HTLV_{CR}$  reverse transcriptase<sup>6</sup>,  $\rm HTLV_{MB}$  reverse transcriptase has a preference for  $\rm Mg^{2+}$  with  $\rm poly(A) \cdot oligo(dT)$  and poly(C)·oligo(dG) (data not shown).

Analysis of isolated core structures from HTLV<sub>CR</sub> has shown that a 24,000 molecular weight protein (p24) is a major component of the viral cores<sup>7</sup>; this protein has been purified<sup>7</sup>. In a competitive radioimmunoassay using labelled, purified  $HTLV_{CR}$  p24 and an antibody against whole disrupted HTLV<sub>CR</sub>, all known animal retroviruses tested to date have failed to compete, whereas purified HTLV<sub>CR</sub> and cells producing HTLV<sub>CR</sub> do compete<sup>7</sup>. Both HTLV<sub>MB</sub> and HTLV<sub>CR</sub> isolates competed in the HTLV<sub>CR</sub> p24 assay (Fig. 4a), suggesting a high degree of relatedness between p24 of the two isolates. Furthermore, cell lysates of cultured, IUdR-induced CTCL-2 T lymphoblasts also compete whereas lysates of normal phytohaemagglutinin (PHA)-stimulated human T lymphoblasts do not (Fig. 4a, b). These findings indicate that p24 is expressed in

Fig. 1 Morphology and karyotypes of CTCL-2 and HUT102 cells. Light microscopic appearance of cultured HUT102 (a) and (b) CTCL-2 cells. Cells were pelleted in a cytocentrifuge and stained with Wright-Giemsa. Both cell lines are morphologically similar, with atypical mono- and multinucleated lymphoblasts. Karyotype analysis was performed as previously described<sup>11</sup>. CTCL-2 metaphases (c) were those of a normal diploid human female (46, XX), while HUT102 metaphases (d) were predominantly those of a pseudodiploid human male (46, XY, minus chromosome 22, plus a minute).



CTCL-2 cells after IUdR induction but not in normal human T lymphoblasts with or without IUdR induction.

Liquid molecular hybridization experiments<sup>5</sup> on HTLV<sub>CR</sub> showed that: (1) HTLV<sub>CR</sub>  $^3$ H-cDNA hybridized almost completely (90%) to its own 70S RNA with kinetics consistent with the genetic complexity of other retroviruses, (2) HTLV<sub>CR</sub> was not significantly related to a wide variety of animal retroviruses (types B, C and D), including bovine leukaemia virus (BLV) and previously described endogenous and horizontally transmitted viruses from non-human primates, and (3) nucleic acid sequences related to  $^3$ H-cDNA or  $^{125}$ I-70S RNA HTLV<sub>CR</sub> were not found in DNA from several normal human tissues.

In liquid hybridization experiments to assess the nucleotide sequence relatedness of HTLV<sub>CR</sub> and HTLV<sub>MB</sub>, 90% of the <sup>3</sup>H-cDNA from HTLV<sub>CR</sub> hybridized to HTLV<sub>CR</sub> 70S RNA and 54% to RNA from cultured HUT102 cells. In contrast, there was no significant hybridization (<10%) to RNA from phytohaemagglutinin (PHA)-stimulated human peripheral blood T lymphocytes from 20 pooled normal donors, to RNA from a normal human embryonic cells line, or to 70S RNA from several other type-C viruses (Table 1). As with cytoplasmic RNA of HTLV<sub>CR</sub>-producing cells, <sup>3</sup>H-cDNA prepared from HTLV<sub>CR</sub> also hybridized to IUdR induced CTCL-2 cytoplasmic RNA (45%). The kinetics of hybridization indicate that CTCL-2 cytoplasmic RNA contains about 0.5% HTLV-specific sequences by weight (Fig. 5). The T<sub>m</sub>s of the resultant hybrids are identical with both sets of cell RNA (Fig. 5 inset).

 $^3\text{H-cDNA}$  from purified HTLV<sub>MB</sub> hybridized 43% to HTLV<sub>CR</sub> 70S RNA but only 19% to 70S RNA from avian myeloblastosis virus (AMV), providing further evidence that HTLV<sub>MB</sub> is closely related to HTLV<sub>CR</sub>. (The 19% value with AMV 70S RNA is indicative of a high background with this cDNA, not a specific relatedness to AMV.) As we had insufficient HTLV<sub>MB</sub> to prepare 70S RNA, we cannot say whether 43% represents the maximum ability of this cDNA to hybridize to the 70S RNA of HTLV<sub>CR</sub> or whether the HTLV<sub>MB</sub> and HTLV<sub>CR</sub> have some nonprelated sequences. The data clearly show, however, that the two isolates are at least closely related. This relationship is now being investigated further.

A frozen portion of the original (uncultured) neoplastic peripheral blood cells used to establish cell line CTCL-2 from patient M.B. was examined for the presence of HTLV<sub>CR</sub>-related

proteins and nucleic acid sequences. Proteins from cell lysates of these fresh cells competed effectively in the radioimmunoassay for HTLV<sub>CR</sub> p24, whereas extracts from PHA-stimulated normal human T lymphoblasts did not (Fig. 4b). Cell proteins were fractionated by phosphocellulose column chromatography as described elsewhere and the fractions eluting from a NaCl gradient at 150–300 mM were positive for HTLV p24 as assessed by competition with  $^{125}\text{I-p24}$  of HTLV in competition radioimmunoassays (Fig. 4b). These results indicate that a protein antigenically related or identical to HTLV<sub>CR</sub> p24 was

Table 1 Nucleic acid relatedness of retrovirus isolates,  $HTLV_{CR}$  and  $HTLV_{MB}$ 

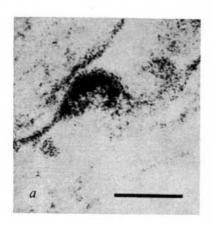
Nucleic acid	% Hybridization with
RNA	
Viral	
HTLV <sub>CR</sub> 70S	90
GALV <sub>H</sub> 70S	13
BaEV 70S	7
AMV 70S	4
Cellular	
HUT102 (HTLV <sub>CR</sub> )	54
CTCL-2 (HTLV <sub>MB</sub> )	45
PHA-stimulated normal human lymphoblasts	9
Human embryonal carcinoma cells	6
DNA	
HUT102 (HTLV <sub>CR</sub> )	45
CTCL-2 (HTLV <sub>MB</sub> )	21
Fresh leukaemic cells of patient M.B.	17
PHA-stimulated normal human lymphoblasts	8
Normal human tissues	3-11*

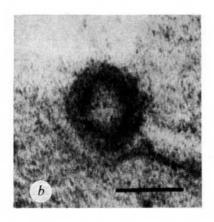
HTLV  $^3$ H-cDNA (500–1,000 c.p.m.) was hybridized to 1  $\mu$ g 70S viral RNA, 200–300  $\mu$ g cellular cytoplasmic RNA or 600  $\mu$ g cellular DNA as described previously  $^4$ - $^{20}$ , and the per cent hybridization determined by S<sub>1</sub> nuclease digestion. The values indicated are maximum plateau values in the conditions used. The heterologous viral 70S RNAs tested included those from gibbon ape leukaemia virus, Hall's Island strain (GALV $_{\rm H}$ ) $^{22}$ , baboon endogenous virus (BaEV) and avian myeloblastosis virus (AMV). The cell lines HUT102 and CTCL-2 are the abnormal T-lymphoblast cell lines derived from patients C.R. and M.B., respectively. The viruses produced by these two cell lines are shown in parentheses. PHA-stimulated lymphoblasts are human peripheral blood leukocytes pooled from 20 normal donors and stimulated in short-term culture (72 h) with PHA prepared as previously described<sup>23</sup>

<sup>\*</sup> Mean of 30 samples = 8%, s.d. = 4%.

present in the original uncultured tumour cells. The IUdR-induced cultured CTCL-2 cell lysates competed at a lower protein input (Fig. 4a) than the fresh lysate (50% competition for cultured cell lysate at 0.8  $\mu$ g protein compared with 150  $\mu$ g for the fresh cells), indicating a higher p24 concentration in cultured cells.

 $\rm HTLV_{CR}$   $^3\rm H\text{-}cDNA$  hybridizes to DNA from both cultured  $\rm HUT102$  (45%) and CTCL-2 (21%) cells, but not to DNA from normal human tissues nor to DNA from a human embryonal carcinoma cell line (Table 1). In addition,  $\rm HTLV_{CR}$  cDNA hybridized 17% to DNA from the original fresh peripheral blood neoplastic T cells of patient M.B. (Table 1), whereas hybridization of  $\rm HTLV$   $^3\rm H\text{-}cDNA$  to DNA of normal human tissues was 3–11% (>30 samples tested), mean  $8\pm4$  s.d. The differences between the degree of hybridization of  $\rm HTLV_{CR}$  cDNA to DNA of cultured cells from patient C.R. and DNA of





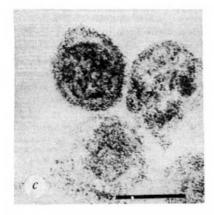


Fig. 2 Electron micrographs of HTLV<sub>MB</sub>. Thin section electron microscopy was performed on CTCL-2 cell pellets as previously described. Shown here are typical budding (a) and extracellular immature (b) and mature (c) type C particles found in clumps adjacent to the T lymphoblasts. Scale bar, 100 nM.

Strain from C.R.

Strain from M.B.



# P56 Constitutive

# Fresh lymphocytesIDUR

#### 3. CTCL-3

P2 Constitutive

Fig. 3 Schematic representation of HTLV isolation from the fresh and cultured cells from two patients (C.R. and M.B.) with cutaneous T-cell lymphoma and leukaemia, respectively. The dots indicate that the cell lines continue to produce virus in succeeding culture passages. The p numbers refer to different passages of the cells in culture. IUdR refers to requirement of the cells for treatment with IUdR for release of HTLV, while 'constitutive' indicates that the cells either subsequently or originally produced HTLV without IUdR (see text for further details).

fresh and cultured cells from patient M.B. again suggest that some non-related nucleic acid sequences exist in the HTLV isolates. The specificity of this hybridization is emphasized by the fact that <sup>3</sup>H-cDNA from Simian Sarcoma virus, baboon endogenous virus, BLV, Rauscher murine leukaemia virus, squirrel monkey virus and langur endogenous virus failed to hybridize to CTCL-2 cytoplasmic RNA (data not shown). The hybridization of HTLV<sub>CR</sub> cDNA to DNA from the fresh Sézary T cells was significantly less than with DNA from cell lines

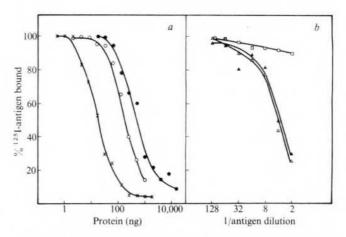


Fig. 4 Relatedness between p24 from HTLV<sub>CR</sub> and from HTLV<sub>MB</sub> and evidence for this p24 in fresh blood cells of patient M.B. HTLV p24 was purified by a combination of phosphocellulose chromatography and gel filtration in Biogel p60 as described earlier<sup>7</sup>. The proteins were iodinated by the chloramine-T method. The radioimmunoassays used 7,000 c.p.m. of <sup>125</sup>I-labelled HTLV p24 and a limiting dilution of a rabbit antibody to HTLV<sup>7</sup>. The viruses and cells used in the assays were solubilized in buffer containing 10 mM phosphate pH 7.5, 0.8 M NaCl, 0.5% Triton X-100 and 0.5 mM phenyl methyl sulphonyl fluoride. The competing unlabelled antigens used in the assay are: (in panel a) ×, HTLV<sub>CR</sub>; ○, HTLV<sub>MB</sub> from cultured CTCL-2 cells; ♠, extracts from cultured CTCL-2 cells; and (in panel b) △, fresh blood cells of patient M.B.; ♠, pool of the proteins from fresh blood cells of patient M.B. partially purified by phosphocellulose chromatography; □, extracts from cultured normal human T cells.

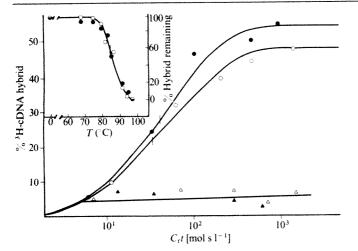


Fig. 5 Nucleic acid relatedness of HTLV<sub>MB</sub> to HTLV<sub>CR</sub>. HTLV<sub>CR</sub>  $^3$ H-cDNA was prepared as previously described $^5$ , hybridized to 200  $\mu g$  of the indicated cytoplasmic RNA in 50% formamide-3X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7) with 0.05% SDS and 1 mM diethylpyrocarbonate, and the amoun of hybrid assayed by S<sub>1</sub> nuclease digestion. The cDNA was hybridized to the indicated  $C_r t$ . Values were normalized to the maximum value with HTLV 70S RNA (actual value 90%). Inset: cDNA was hybridized to a C,1 of 500, diluted 20-fold with 2X SSC, and incubated at the indicated temperature for 5 min. Hybridization is normalized to 100% for each hybrid (actual values 48% and 44%, respectively). •, HUT102 cytoplasmic RNA; ○, IUdR-treated CTCL-2 cytoplasmic RNA; △, human embryonic cell line cytoplsmic RNA; A, PHA-stimulated normal human blood T lymphoblasts obtained as previously described<sup>23</sup>.

infected by and producing HTLV. These findings are consistent with the presence of HTLV proviral sequences in DNA of a fraction of the primary tumour cells and/or the presence of only fractions of the provirus in most tumour cells.

Thus, the present data indicate that an isolate of a type C retrovirus (HTLV<sub>MB</sub>) from the cultured leukaemic cells of a patient with Sézary syndrome is closely related to HTLV<sub>CR</sub> from a patient with T-cell lymphoma, by virtue of their structural protein and nucleic acid sequence homology. Further studies are required to identify any differences between the two isolates. The observations that the HTLV isolates are not ubiquitous genetically transmitted viruses of man<sup>5</sup>, that HTLV-related nucleic acid sequences and proteins are found in fresh leukaemic blood cells of patient M.B. and that specific antibodies to HTLV occur in some human sera<sup>16.17</sup> indicate that HTLV has infected some humans. The results also suggest that its provirus was integrated into the human genome of at least some of the leukaemic cells. Consistent with these findings, recent electron microscopic studies of van der Loo et al. show apparent retrovirus-like particles in primary (uncultured) tumour cells of a patient with cutaneous T-cell lymphoma<sup>18</sup>

Many animal retroviruses are known to cause leukaemias and lymphomas of T cells in their host 19. Particularly interestingly, a cutaneous lymphoma can occur in sheep after inoculation with BLV<sup>20,21</sup>. HTLV has so far been found only in the neoplastic tissue of three patients with T-cell malignancies. Seroepidemiological studies and surveys of cell nucleic acids for HTLV sequences are in progress to obtain further evidence for a possible relationship of HTLV particles to these diseases.

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# Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus

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Although retroviruses (RNA tumour viruses) have been implicated in the causation of naturally occurring leukaemias and lymphomas of animals1, it is not yet clear whether they are involved in the human versions of these malignant diseases, particularly because of the difficulty in isolating viruses truly ascribable to human origin<sup>2,3</sup>. However, a novel type C retrovirus (called  $HTLV_{CR}$ ) has been isolated in our laboratory from T cells (fresh and in culture) from a lymph node biopsy of a patient (C.R.) with cutaneous T-cell lymphoma (mycosis fungoides)4 and a very similar virus (HTLV<sub>MB</sub>) from the peripheral blood T cells of another patient (M.B.) with cutaneous T-cell leukaemia (Sézary syndrome, see accompanying report<sup>5</sup>). The nucleic acid sequence<sup>6</sup>, the reverse transcriptase and the major internal structural protein (p24) of HTLV<sub>CR</sub> are not significantly related to any of the known retroviruses; nucleic acid sequences and p24 protein of HTLV<sub>MB</sub> have been recognized in fresh and cultured cells<sup>5</sup>. We now describe the results of a limited survey of the occurrence, in patients with T-cell malignancies (and among normal people), of antibodies against HTLV<sub>CR</sub> proteins. We find that antibodies against p24 are present in human sera (including those of patient C.R. and his wife), and that these are specifically directed at HTLV<sub>CR</sub> proteins and not at cell-specific determinants—in other words, the immunological reactions are not those reported in human sera<sup>9-11</sup> against animal virus glycoproteins which, lacking virus specificity, are directed against the carbohydrate residues of the glycoprotein<sup>12,13</sup>. The antibodies against HTLV are thus the first evidence for a specific immune response in humans against a retrovirus.

A limited survey was designed to determine whether natural antibodies reactive against the human retrovirus isolate, HTLV, can be detected in human sera. The assay used in this preliminary survey was a radioimmune precipitation of 125I-labelled HTLV<sub>CR</sub> p24 by human sera using a double antibody system<sup>8</sup>. The p24 of HTLV<sub>CR</sub> was purified to homogeneity from density-

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Table 1 Specificity of precipitation of HTLV<sub>CR</sub> p24 by natural antibodies in human sera

125Y labelled antique			Per cent competition by										
125I-labelled antigen used in RIA	Serum	Diagnosis	HTLV <sub>CR</sub>	R-MuLV	FeLV	SSV	BaEV	MPMV	SMRV	BLV			
HTLV <sub>CR</sub> p24	Patient C.R.	Cutaneous T-cell lymphoma	98	3	6	4	3	5	0	2			
	Patient M.J.	Cutaneous T-cell leukaemia	93	2	0	0	1	0	3	0			
	Wife of patient C.R.	Normal	90	0	1	4	9	5	9	2			
R-MuLV gp70	M.R.G.	Normal	100	100	100	100	97	100	100	100			
<u>.</u>	Patient S-924	Chronic myelogenous leukaemia	72	88	83	80	68	78	79	84			

Competition radioimmunoassay (RIA) was performed as described in Fig. 2 legend using the competing viruses at 10 µg protein. Buffer 1 was used in the precipitations of <sup>125</sup>I-labelled HTLV<sub>CR</sub> p24 and buffer B in the precipitations of <sup>125</sup>I-labelled R-MuLV gp70. FeLV, feline leukaemia virus, SSV, simian sarcoma virus, BaEV, baboon endogenous virus; MPMV, Mason-Pfizer monkey virus; SMRV, squirrel monkey retrovirus; BLV, bovine leukaemia virus.

banded virus and radiolabelled with  $^{125}I$  as described elsewhere<sup>8</sup>. The serum from patient C.R. precipitated >90% of the labelled p24, and the serum of another patient (M.J.) with Sézary syndrome showed a similar high reactivity towards HTLV<sub>CR</sub> p24. Twenty-one other serum samples from various T-cell malignancies and 50 sera from random normal donors showed no significant precipitating activity. Sera were also obtained from 11 close family members of patients with T-cell malignancies, with previous evidence of HTLV infection. Out of these, one serum (no. 81-5, from the wife of patient C.R.) reacted strongly in the immunoprecipitation.

The reactivity of the positive sera was characterized in detail, and Fig. 1 shows the pattern of precipitation obtained. The radiolabelled probe used was 95% immunoprecipitable with a hyperimmune rabbit serum against disrupted HTLV<sub>CR</sub>, whereas sera 38-7 and 24-1 from patients C.R. and M.J. respectively precipitated >80% and the apparently normal serum 81-5 precipitated >50% of the  $^{125}$ I-labelled p24 at the highest serum concentration tested. Thus, the two highly positive human sera had antibody titres very similar to that of the hyperimmune rabbit serum. The profile of normal serum M.R.G. in Fig. 1 is typical of the results obtained with all the negative sera tested. These results concur with data obtained in an independent study using solid phase radioimmunoassay which showed specific reactivities in these sera towards HTLV<sub>CR</sub> (ref. 14).

reactivities in these sera towards HTLV<sub>CR</sub> (ref. 14).

In view of the past controversy<sup>15</sup> concerning the presence of natural antibodies in human sera reactive with antigens from animal retroviruses, the reactivities shown in Fig. 1 were analysed for their specificity. Various retroviruses and cell

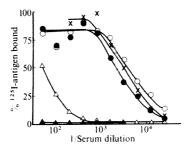


Fig. 1 Immunoprecipitation of <sup>125</sup>I-labelled HTLV<sub>CR</sub> p24 by different human sera. HTLV<sub>CR</sub> p24 was purified and labelled with <sup>125</sup>I as described elsewhere<sup>8</sup>. The labelled p24 (8,000–10,000 c.p.m.) was mixed with two-fold serial dilutions of human sera or a hyperimmune rabbit serum raised against HTLV in a volume of 20 μl of buffer 1 (200 mM Tris-HCl, pH 7.5, 200 mM NaCl, 1 mM EDTA, 0.3% Triton X-100, 0.1 mM phenylmethylsulphonyl fluoride and 2 mg ml<sup>-1</sup> bovine serum albumin). The reaction mixture was incubated for 2 h at 37 °C and overnight at 4 °C. A 20-fold excess of goat anti-human IgG (or goat anti-rabbit IgG when rabbit IgG was the primary antibody) was then added and the volume made up to 500 μl with buffer 1. The samples were incubated for 1 h at 37 °C and an additional 2 h at 4 °C and then centrifuged at 2,500 r.p.m. for 15 min in a Beckman centrifuge. The supernatants were aspirated and the radioactivity in the pellets was counted in an LKB Ultrogamma counter. ♠ Human serum (patient C.R.); ♠, human serum (patient M.J.); △, human serum (wife of patient C.R.); ♠, normal human serum, M.R.G.; ×, rabbit antiserum to HTLV<sub>CR</sub>.

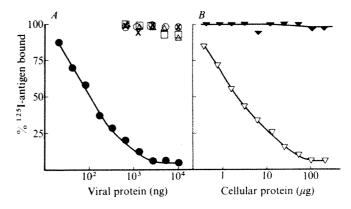


Fig. 2 Specificity of the precipitation of HTLV<sub>CR</sub> p24 by serum of patient M.J. Competition radioimmunoassays were set up using <sup>125</sup>I-labelled HTLV<sub>CR</sub> p24 and a limiting dilution (1:7,500) of the human serum (patient M.J.). Serial dilutions of the unlabelled antigens were preincubated with the serum for 1 h at 37 °C. Labelled p24 (8,000-10,000 c.p.m.) was then added and the reaction mixture incubated and processed as described in Fig. 1 legend. a, Competition by retroviral extracts: ♠, HTLV<sub>CR</sub>; ○, SSV; ×, BaEV; △, SMRV; □—□, BLV. b, Competition by cell extracts: ▽, HUT102 cells producing HTLV<sub>CR</sub>; ▼, HUT78 cells.

extracts were tested for their capacity to compete with the precipitation of <sup>125</sup>I-labelled p24 induced by the positive human sera. Figure 2 shows results obtained with the serum of patient M.J. Simian sarcoma virus, baboon endogenous virus, Mason-Pfizer monkey virus and bovine leukaemia virus failed to neutralize the reactivity of the serum for p24. Only HTLV<sub>CR</sub> effectively competed in the precipitation (Fig. 2a). Similarly, extracts from cells which produce HTLV<sub>CR</sub> totally competed in the precipitation, whereas extracts from a neoplastic human T-cell line (HUT78) which is negative for HTLV<sub>CR</sub> (ref. 8) failed to compete (Fig. 2b). This specificity is very similar to that observed in the precipitation of HTLV<sub>CR</sub> p24 by a hyperimmune serum against disrupted HTLV<sub>CR</sub> (ref. 8). The same strict specificity was observed with all the human sera which showed an immune reactivity with HTLV<sub>CR</sub> p24 (Table 1). Similarly, identical results were obtained whether the immune complex was precipitated with goat anti-human IgG or by the addition of inactivated Staphylococcus aureus cells, indicating that the reactive species in the human sera are intact immunoglobulin molecules.

The extent of immune precipitation of  $^{125}$ I-labelled p24 by the human sera was not affected by the assay conditions used, unlike the situation encountered with human natural antibodies reported to be reactive to envelope glycoproteins of animal retroviruses (refs 12, 13 and data below). The extent and pattern of precipitation of  $HTLV_{CR}$  p24 by human sera were identical, irrespective of whether the immune precipitation medium was supplemented with ovalbumin or bovine serum albumin as a protein carrier (Fig. 3a,b). In contrast, many human sera exhibit strong precipitating activity towards Rauscher murine leu-

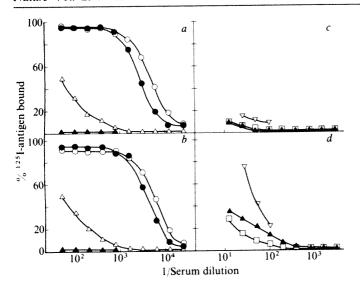


Fig. 3 Effect of buffer composition on the precipitation of <sup>125</sup>I-labelled HTLV<sub>CR</sub> p24 and <sup>125</sup>I-labelled R-MuLV gp70 by human sera. Immuno-precipitation of <sup>125</sup>I-labelled HTLV<sub>CR</sub> p24 and <sup>125</sup>I-labelled R-MuLV gp70 by the human sera subsequence of the first part of the first bovine serum albumin and 0.5% Triton X-100) or in buffer B (buffer A containing 5 mg ml<sup>-1</sup> ovalbumin instead of bovine serum albumin). Labelled antigen (8,000-10,000 c.p.m.) was incubated with twofold serial dilutions of the human sera in 200 µl of the appropriate buffer and the extent of immune precipitation measured as described in Fig. 1 legend. a, b Represent prereplation of HTLV<sub>CR</sub> p24 in buffer A and buffer B respectively. •, Serum of patient C.R.; O, serum of patient M.J.; △, serum of the wife of patient C.R.; A, normal serum. c, d Represent precipitation of R-MuLV gp70 in buffer A and buffer B, respectively. 

¬, serum of chronic myelogenous leukaemia patient, 7-117; 

¬, serum of patient with chronic myelogenous leukaemia, S-964; A-A, normal human serum, M.R.G.

kaemia virus (R-MuLV) gp70 when the reaction is done in the presence of ovalbumin as the carrier (Fig. 3d). This activity is nearly eliminated if bovine serum albumin is used instead of ovalbumin (Fig. 3c). Such reactivity is also observed with gp70s of other retroviruses and is thought to be directed, not against the polypeptide portions of the gp70 that are virus coded, but against the carbohydrate moieties on the molecule that are introduced by the host cell as a post-transcriptional event, and which are therefore cell-specific and not virus-specific 12,13. This lack of viral specificity is clearly shown by the finding that, unlike the activity towards  $HTLV_{CR}$  p24, all the viruses tested competed totally in the precipitation of R-MuLV gp70 (Table 1).

We have described here a specific immune reactivity in some human sera towards the internal structural antigen p24 of HTLV<sub>CR</sub>, a type C retrovirus, isolated from the T cells of two different patients with T-cell malignancies. Of 84 sera tested, 3 have shown significant reactivity towards this viral antigen. It is possible that other sera contain anti-HTLV<sub>CR</sub> antibodies but were missed because the assay detects only antibodies to the HTLV<sub>CR</sub> core protein, p24. In this regard, use of an envelope antigen of the virus as the probe may show a more representative frequency of natural antibodies in humans towards the virus. These and independent tests using solid phase radioimmunoassay (ref. 14) are the first evidence of a specific immune response to a type C retrovirus in humans. As animal retroviruses can cause T-cell leukaemias and lymphomas, the finding of these antibodies only in sera of people with T-cell neoplasias and in the positive normal case (the wife of the patient from whom HTLV was first isolated), combined with other recent evidence for the presence of this virus in some of these patients, warrants careful consideration of a possible role for HTLV in the origin of these kinds of human leukaemias and lymphomas.

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# The *v-sis* transforming gene of simian sarcoma virus is a new onc gene of primate origin

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The defective transforming simian sarcoma virus (SSV) and its nondefective helper virus (SSAV) are retroviruses isolated from a fibrosarcoma of a pet woolly monkey. Together with the gibbon ape leukaemia viruses, they are the only group of retroviruses known to cause spontaneous and experimentally induced neoplasia in primates (see refs 1 and 2 for review). Molecular cloning has shown that SSV contains a 1.2-kilobase (kb) transformation-specific viral onc gene (v-sis)3,4 which, like other viral onc genes, is derived from a set of conserved cellular DNA sequences<sup>5</sup>. A human DNA fragment containing sequences homologous to the entire v-sis gene has also been cloned and analysed. We present here experiments carried out on the cloned SSV genome which show that: (1) v-sis is distinct from other viral transforming (onc) genes; and (2) v-sis is derived from a woolly monkey naturally infected once with gibbon ape leukaemia virus (GaLV) and is to date the only onc gene of primate origin.

Different viral onc genes have been known to share a common cellular progenitor. There are many examples of viruses isolated from the same species acquiring the same onc gene, and at least one example of a cellular one gene represented in viruses from two different species (chicken and cat)<sup>7</sup>. To test possible homology between v-sis and other viral onc genes, we digested DNA from a recombinant phage clone (C60) of SSV with the restriction endonucleases BglII or a combination of SalI and PvuII to localize the v-sis sequences on a gel, which we blothybridized8 to 32P-labelled plasmid clones containing onc sequences from different acutely transforming retroviruses. Table 1 summarizes the origin and properties of the probes used. The detailed restriction map of C60 has been published elsewhere<sup>3</sup>. This clone has two copies of the long terminal repeat (LTR) and an inversion involving one LTR and 0.1 kb of adjacent viral sequences. In each case Bg/II or SalI and PvuII generated a 0.65-kb fragment that was highly enriched in v-sis sequences, as demonstrated by the poor detection of this

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Table 1         Origin and properties of probes								
Viral origin	Ref.	pBR322 sequences	onc sequences (kb)	% Of complete onc genes	Helper sequences (kb)			
SSV	3	+	1.2	100	4.8			
SSAV	3	+	0	ND	9.0			
HaMSV	14	+	1.0	100	5.2			
KiMSV	15	+	?	100	?			
M-MSV	16	Trans.	0.8	60	0			
A-MuLV	17	+	2.5	60	0			
FeSV <sub>ST</sub>	18	+	1.2	85	0			
ASV	19	+	1.5	100	8.5			
MC29	20	+	2.0	100	0.8			
AMV	21	+	0.6	100	1.9			

SSAV, simian sarcoma associated virus; HaMSV, Harvey murine sarcoma virus; KiMSV, Kirsten murine sarcoma virus; M-MSV, Moloney murine sarcoma virus; A-MuLV, Abelson murine leukaemia virus; FeSV<sub>ST</sub>, Snyder-Theilen strain of feline sarcoma virus; ASV, avian sarcoma virus, Schmidt-Ruppin A strain; MC29, avian myelocytomatosis virus; AMV, avian myeloblastosis virus. ND, not determined.

fragment by the SSAV probe compared with the homologous SSV probe (Fig. 1a, b; and see simplified map of C60). None of the other viral onc probes detected the 0.65-kb fragment. Except for Harvey murine sarcoma virus (HaMSV) and Kirsten murine sarcoma virus (KiMSV), the murine or feline probes contained no helper sequences and did not hybridize detectably to any SSV DNA fragment (Fig. 1d). Although the avian viral probes did contain helper sequences, they showed no detectable homology with SSAV-derived sequences (Fig. 1d). The HaMSV and KiMSV plasmids used contained the entire viral genome, and hybridization to the 2.8-kb PvuII fragment or 3.3-kb BgIII fragment of SSV (Fig. 1c) was probably due to cross-reactivity of the LTR sequences of the two viruses.

To ensure that the *onc* genes of HaMSV and KiMSV were indeed unrelated to any portion, especially the 3' region, of *v-sis*, further restriction enzyme analyses were carried out. For these experiments, a clone of SSV (C14) which contained one LTR and no inversion in its genome was used<sup>24</sup>. DNA from C14

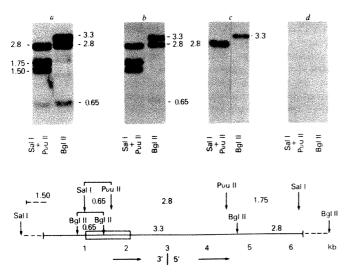


Fig. 1 Lack of homology of v-sis to other viral onc sequences. DNA (40 ng) from a Charon 21A-SSV recombinant phage (C60) was digested with  $Bg\Pi$ I or  $Sa\Pi + Pvu\Pi$  on 1.0% agarose gels and transferred to nitrocellulose filters as described elsewhere  $^6$ . Multiple replicate filters were made and each hybridized to a  $^{32}$ P probe prepared from the cloned DNA after nick translation  $^{23}$ . a, SSV; b, SSAV; c, HaMSV, KiMSV; d, M-MSV $_{onc}$ , FeSV $_{onc}$ : A-MuLV $_{onc}$ : AMV, MC29, ASV(SR). The filters were washed in 1×SSC at 60 °C and autoradiography was carried out for 4–16 h. A simplified map of C60 is shown at the bottom. Sizes are given in kb.

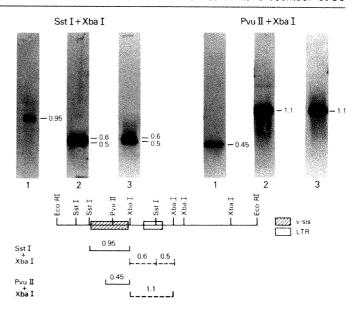


Fig. 2 Assay for homology between a Charon 21A-SSV recombinant phage (C14) and plasmid clones of HaMSV and KiMSV. Triplicate filters containing C14 DNA digested with Sst1 + Xba1 or PvuII + Xba1 were hybridized to <sup>32</sup>P-labelled probes of: (1) p335, a plasmid subclone of the 3' half of a human c-sis gene, (2) HaMSV and (3) KiMSV. A simplified map of C14 indicating the v-sis and LTR sequences is also shown. Sizes are given in kb.

was co-digested with SstI and XbaI or with PvuII and XbaI. These digestions delimited more precisely the boundaries of v-sis and LTR sequences (see map in Fig. 2). To locate more conveniently the 3' region of v-sis, a subclone (p335) derived from a BamHI-BamHI fragment of the human c-sis gene homologous to the 3' two-thirds of v-sis was used as a probe. This clone has no SSAV-related sequences<sup>6</sup>. Replicate filters were hybridized to 32P-labelled pBR322 plasmid clones containing HaMSV and KiMSV genomes. p335 detected a 0.95-kb fragment in the SstI+XbaI digestion and a 0.45-kb fragment in the PvuII+XbaI fragment (Fig. 2), as expected from the map. The HaMSV and KiMSV probes, in contrast, hybridized only to fragments containing LTR sequences (0.6 and 0.5 kb in the SstI+XbaI digest and 1.1 kb in the PvuII+XbaI digest) and not at all to sis-containing fragments. This lack of homology between v-sis and v-Harvey-ras is consistent with our previous demonstration that v-sis and v-Harvey-ras hybridized to distinct sets of sequences in vertebrate DNA<sup>5</sup>. Here we also show that v-sis is distinct from v-Kirsten-ras. Thus, taken together, our results suggested that v-sis was distinct from other viral onc genes. Of particular interest was the lack of homology between v-sis and the M-MSV, HaMSV or KiMSV onc genes. The genetic structure of SSV is most similar to M-MSV in that its gene order is 5'-\(\Delta gag-\Delta env-onc-C-3'\) (ref. 3). Its transformation-specific protein product is a 20,000-molecular weight protein (our unpublished data, and J. Thiel and D. Bolognesi, personal communication), similar in size to the transforming protein (p21) of KiMSV and HaMSV9. Our results suggest that v-sis is distinct from these three rodent-derived onc genes.

All the known viral onc genes are derived from normal host cell sequences that are phylogenetically conserved among vertebrates. Using a non-stringent and sensitive hybridization technique such as Southern hybridization<sup>8</sup>, we have shown that v-sis sequences could detect homologous loci in DNA from chicken to man<sup>5</sup>. Determination of its actual host species of origin, however, would require a more stringent assay of homology. We have subcloned a PstI-PstI fragment of the SSV genome that contains ~250 base pairs (bp) of 5' v-sis sequences and 900 bp of SSAV-derived sequences into the bacteriophage M13. The single-stranded phage DNA was then partially digested with DNase and terminally labelled with <sup>32</sup>P by the kinase

Table 2 Species of origin of p-sis sequences

	S <sub>1</sub> nuclease resistant (c.p.m.)	% v-sis sequences hybridized							
Woolly monkey	11,000	87							
Marmoset	9,200	71							
Gibbon	6,100	54							
Human	5,500	42							
Rat	1,000	8 -							
Cat ,	800	6							

The M13 mp7 recombinant phage was constructed as described previously<sup>22</sup>. The single-stranded phage DNA was purified, 5 µg of the DNA were digested with 0.4 ng DNase I for 15 min at 25 °C, extracted with phenol and dialysed. The partially digested DNA was then treated with bacterial alkaline phosphatase (3 U) at 60 °C for 30 min, extracted with phenol and precipitated in ethanol. The precipitate was collected by centrifugation, and incubated with polynucleotide kinase and 0.2 mCI  $[\gamma^{-32}P]$ ATP (7,000 Ci nmol<sup>-1</sup>) at 37 °C for 30 min. The labelled DNA was purified by three cycles of ethanol precipitation in ammonium acetate buffer. About 400,000 c.p.m. of the labelled probe (~12,000 c.p.m. of v-sis sequences) was hybridized to 500  $\mu$ g of cellular DNA to a  $C_0t$  of >10<sup>4</sup>. Hybridization was monitored by  $S_1$  nuclease resistance as described elsewhere<sup>1</sup>.

reaction<sup>10</sup>. The labelled DNA was hybridized in solution to cellular DNA from tissues of different animals and the resultant hybrids assayed by their resistance to S<sub>1</sub> nuclease<sup>11</sup>. Of all the DNA examined, primate DNA hybridized significantly better than DNA from non-primates (Table 2). Among the primates, the New World monkeys (woolly monkey and marmoset) contained more DNA sequences homologous to v-sis than the Old World apes (gibbon and man) and the highest homology was obtained with woolly monkey DNA. The SSAV genome had no detectable homology to primate DNA either by Southern hybridization<sup>5,6</sup> or liquid hybridization (not shown), and its distant homology to rodent DNA could only be monitored by non-stringent hybridization conditions 12. We conclude that v-sis originated from woolly monkey DNA. The close relatedness of SSAV to different GaLV isolates and the revelation that the woolly monkey that yielded SSV/SSAV co-habited with a gibbon before it developed sarcoma<sup>11</sup> suggest that SSAV was transmitted from the gibbon to the woolly monkey during that period, and that in a single infection event, SSAV had recovered an onc gene from woolly monkey to generate SSV.

Although all viral onc genes thus far identified are conserved among vertebrate species from bird to man, there seems to be some species specificity with respect to the particular onc gene(s) accessible for recombination. For example, the same chicken onc gene (myc) was recovered in four independent recombination events to generate the leukaemia viruses MC29, MH2, CMII and OK10 (ref. 13). The only known example of related cellular onc genes from different species becoming viral onc genes is the relationship between the feline sarcoma virus (Snyder-Theilen and Gardner-Arnstein strains) and avian Fujinami sarcoma virus<sup>7</sup>. Therefore, the genetic arrangement of a particular onc gene may vary between species and may determine its frequency or recombination. As v-sis is the first known one gene derived from a primate, it would be of interest to see whether subsequent primate virus isolates will pick up the same gene.

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# Structure of the glycoprotein gene in rabies virus

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Rables virus; a rhabdovirus, has a single (non-segmented) negative-strand RNA genome which is transcribed on infection to produce five polyadenylated complementary monocistronic mRNA species. Each of the virus-specific mRNAs representing a structural gene of the rables virus genome<sup>1</sup> codes for a virion structural protein which corresponds in size to the apparent coding capacity of its mRNA<sup>2,3</sup>. The glycoprotein gene codes for a membrane-associated molecule which forms spike-like projections on the surface of mature rables virious and is responsible for the induction and binding of virus-neutralizing antibodies to the virus<sup>4-4</sup>. To define the antigenic and immunogenic properties of rables virus glycoprotein, we have cloned a cDNA copy of its mRNA sequence into pBR322 (ref. 7). Here we describe the characterization of the cDNA sequence, which has allowed as to predict several features of the glycoprotein from the deduced amino acid sequence.

The five rabies virus-specific mRNAs for L, G, N, M<sub>1</sub> and M<sub>2</sub> proteins have been characterized by sucrose gradient3 and gel analysis<sup>2</sup>: L mRNA sediments at >28S, glycoprotein (G) mRNA at 18S, nucleocapsid (N) mRNA at 16S and matrix (M1 and M<sub>2</sub>) mRNAs at 12S. For the cloning of the G mRNA, RNA extracted from rabies virus-infected BHK cells was purified by oligo(dT) cellulose chromatography and sucrose density centrifugation. Poly(A) RNA of ~15-20S was used as starting material (thus eliminating the mRNAs for L, M1 and M2 proteins) for the synthesis of double-stranded complementary DNA. Synthesized DNA was inserted into pBR322 at the PstI site by dG-dC tailing (see Fig. 1 legend). Approximately 1% of the tetracycline-resistant colonies screened using 32P-labelled rables virion RNA responded with varying intensities. From 100 colonies that were re-screened, the 20 giving the strongest signals were selected. The size of the plasmids was checked by digestion with BamHI to give linear molecules and with PstI to excise the insert. About half the plasmids contained an additional BamHI site (group A) while the others contained a single BamHI site (group B). This suggested the presence of two different sequences, which we presumed coded for glycoprotein and nucleocapsid protein.

To distinguish between the putative glycoprotein and nucleocapsid protein mRNA-specific plasmids, the insert of a plasmid from each group was isolated by PstI digestion and

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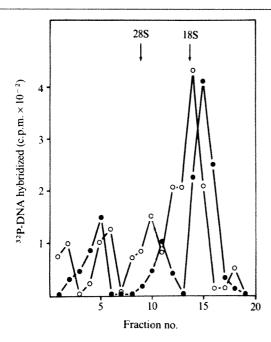


Fig. 1 Hybridization analysis of rabies virus-infected cell poly(A) RNA with rabies recombinant plasmids. Plasmids were constructed as follows: 15-20S poly(A) RNA from rabies virus (ERA strain)-infected cells was transcribed into cDNA using oligo(dT) as primer and AMV reverse transcriptase (supplied by J. Beard), followed by synthesis of double-stranded (ds) cDNA with Escherichia coli DNA polymerase<sup>17</sup>. After S<sub>1</sub> nuclease treatment, ds-cDNA was elongated with dCMP residues by deoxynucleotidyl terminal transferase, and ds-cDNA >1 kbp was selected by sucrose density centrifugation. ds-cDNA tailed with dCMP was hybridized to pBR322 cut with PstI and tailed with dGMP residues, and the recombinant plasmid used to transform E. coli x 1776 (ref. 18). Colonies containing rabies virus-specific sequences were selected by colony hybridization<sup>19</sup> using rabies virion RNA labelled with <sup>32</sup>P by polynucleotide kinase after partial alkali hydrolysis. For hybridization analysis, poly(A) RNA (~130 µg) was heatdenatured at 100 °C for 1 min and centrifuged in a 5-23% sucrose gradient containing 50 mM Tris-HCl pH 7.5, 5 mM EDTA at 25,000 r.p.m. at 10 °C for 16 h in a Beckman SW41 rotor. Each fraction (0.5 ml) from the gradient was diluted 1:60 with water and 1 µl was hybridized with labelled insert (~3,000 c.p.m., specific activity 10<sup>8</sup> c.p.m. µg<sup>-1</sup>) prepared from plasmids A and B. Before hybridization, the probes were heated at 100 °C for 5 min, adjusted to 50% formamide, 0.75 M NaCl, 10 mM HEPES buffer (pH 6.8), 1 mM EDTA, 1 mg ml<sup>-1</sup> yeast RNA, and heated with fractionated poly(A) RNA from rabies virus-infected cells in a total volume of 10 µl at 45 °C for 16 h in paraffin oil. Hybridization was stopped by addition of cold 0.25 M NaCl, 0.03 M sodium acetate (pH 4.5), 1 mM ZnCl<sub>2</sub>. After removal of paraffin oil, S<sub>1</sub> nuclease (100 units) was added and the solution incubated at 37 °C for 45 min. Acid-insoluble counts were determined by trichloroacetic acid precipitation. ○, B; ●, A.

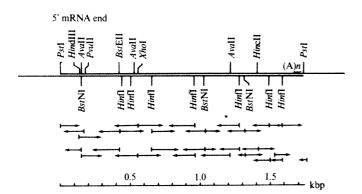
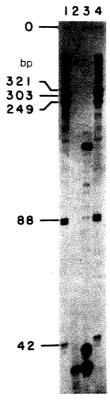


Fig. 2 Restriction map of the insert of pRG. The map was constructed from the size of DNA fragments arising from digestion with combinations of restriction enzymes and estimated by agarose or acrylamide gel electrophoresis 10. The upper set of arrows indicates regions and directions sequenced from the cDNA strand. At the end of the cDNA sequence corresponding to the 5' terminus of the mRNA, there is an oligo(dG-dC) tract of 24 residues with an oligo(dG-dC) tract of 14 residues at the opposite end. Also present at this end is a sequence of poly(dA) which exists in our clone as two lengths of ~50 and 80 bp.

electrophoresis on 1.5% agarose gel<sup>8</sup>. Each insert was labelled by nick-translation<sup>9</sup> and hybridized to individual fractions of poly(A) RNA derived from rabies virus-infected cells and sedimented in a sucrose gradient (Fig. 1). The insert of a group B plasmid hybridized to a mRNA sedimenting at 18S, the location of maximal mRNA activity for the synthesis of glycoprotein, while the insert of group A hybridized to a mRNA sedimenting at 16S, the location of mRNA capable of directing the synthesis in oocytes of nucleocapsid protein (see Fig. 1 in ref. 3). Thus the plasmids of groups A and B were designated pRN and pRG respectively.

Restriction mapping established the size of the inserted DNA of pRG to be ~1.75 kilobase pairs (kbp; Fig. 2). To determine the number of nucleotides which might be missing from the glycoprotein cDNA insert, a small labelled fragment located close to the 5' end of the glycoprotein sequence and bounded by restriction sites for *PvuII* and *HindIII* was hybridized to 18S poly(A) RNA from rabies virus-infected cells. Incubation of the hybrid formed between glycoprotein mRNA and labelled DNA



Comparison of lengths of the glycoprotein cDNA clone and its mRNA. The glycoprotein gene sequence was restricted with PvuII, labelled by  $[\gamma^{-32}P]ATP$  with polynucleotide kinase, and subsequently digested with HindIII. The 5'-labelled 36-bp fragment located close to the 5' terminus of the glycoprotein sequence was isolated on a 5% polyacrylamide (0.1% bisacrylamide) gel in 50 mM Tris-borate pH~8.3,~1 mM EDTA, eluted and dried. The  $^{32}$ P-labelled fragment was then denatured in 20  $\mu$ l of 80% (v/v) formamide by heating at 100 °C for 3 min. A sample of 18S poly(A) RNA (10 µg) from rabies virus-infected cells was dried down with 20 µl of solution containing 0.4 M NaCl, 20 mM HEPES buffer pH 6.8, and 1 mM EDTA pH The solution of denatured 32P-labelled DNA was added to the dried RNA and incubated at 60, 58, 56 and 54 °C for 1 h at each temperature. After annealing, the mixture was transferred to 100 µl cold 0.3 M sodium acetate pH 5.5 and precipitated by 2.5 vol ethanol at -20 °C. The precipitated nucleic acids were dissolved in 40  $\mu$ l of 50 mM Tris-HCl pH 8.3, 10 mM MgCl<sub>2</sub>, 35 mM KCl, 30 mM  $\beta$ -mercaptoethanol, 0.5 mM each of the four deoxynucleoside triphosphates, 200 µg ml<sup>-1</sup> bovine serum albumin, 12.5 µg ml<sup>-1</sup> actinomycin D and AMV reverse transcriptase (12 U), and incubated at 37 °C for 90 min. After precipitation from the reaction mixture with ethanol, the nucleic acids were dissolved in 4  $\mu$ l 80% formamide, 50 mM Tris-borate pH 8.3, 1 mM EDTA, 0.1% xylene cyanol FF and 0.1% Bromophenol blue and heated at 90 °C for 1 min before loading on to a 8% polyacrylamide (0.4% bisacrylamide) gel containing 50% (w/v) urea, 100 mM Tris-borate pH 8.3, 2 mM EDTA. Labelled nucleic acids were detected by autoradiography using an intensifying screen at -70 °C. Lanes 1 and 4, labelled size markers; lane 2, labelled 36-bp fragment bounded by Pvull and HindIII; lane 3, labelled 36-bp fragment elongated by AMV reverse transcriptase. O, origin.

Fig. 4 DNA sequence of the mRNA sense strand and the deduced amino acid sequence for rabies virus glycoprotein (ERA Numbers strain). parentheses refer to the amino acids counted from N-terminal lysine the residue of the mature glycoprotein located 20 amino acids from the presumed initiation codon. A shaded hydrophobic domain close to the C-terminal end is presumed to be the transmembrane segment. Asterisks mark three possible carbohydrate attachment sites based on the presence of Asn-X-Ser and Asn-X-Thr as described in the text. Boxes show the initiation codon (ATG) and termination codon (TGA).

																		AGGA	MG
मुद्	611 val	CC!	CAG gì n	CCT ala	CTC leu	CTS leu	TTT phe	GTA val	OCC PPTO	CTT low	CTG leu	GT T Wal	TTT phe	CCA pro	TTG leu	TGT Cys	TTT phe	g: y	MA lys (1)
TTC	CC T pro	ATT 11e	TAC Lyr	AC G thr	ATA 110	CTA leu	GAC #SP	MG lys (10)	leu	gi y	CCC pro	TGG trp	AGC SOT	ero CCG	ATT ile	GAC esp	ATA 11e	CAT his (20)	his
CTC 100	AGC 1817	TGC CY1	CCA pro	MAC MER	AAT asm	17G leu	GTA val	616 481 (30)	glu	GAC #SP	gaa glu	GGA 91 y	TGC Cys	ACC Uhr	AAC asn	CTG teu	TCA Ser	91 y (40)	phe
TCC	TAC Cyr	ATG met	GAA gl u	CTT leu	AAA Iye	ett val	GA gly	TAC (30)	110	TTA low	ecc ala	ATA 11e	AAA lys	ATG ##t	AAC asn	gi y	TTC	ACT thr (60)	cys
ACA thr	esc ply	et i	GTG val	ACG thr	åj n eve	at a	gaa glu	ACC thr (70)	UT	ACT thr	AAC asn	TTC	GIT val	QGT giy	TAT tyr	A9 J	ACA thr	ACC thr (80)	thr
TTC	MA lys	***	AAG Tys	CAT	TTC	CEC	CCA pro	ACA thr (10)	800	ga t esp	CCA ele	CAR	AGA AF 9	al a	at a	TAC Lyr	***	TGG trp (100)	lys
ATG	a) e	gi y	GAC est	OCC.	AGA MTS	tat Cyr	glu	glu (110)	30"	CTA leu	CAC his	ast ast	<b>b</b> a.o	TAC Lyr	CCT pro	es p	tyr	CGC arg (120)	trp
CTT :	(EA	ACT thr	eta Vat	lys	ACC thr	ACC thr	lys	130) 9) u	107	CTC leu	GTT	ATC fle	ATA 11e	TCT SMT	OEA pro	AGT MAT	44	GCA ela (140)	450
TTG :	es.c	CCA pro	TAT Lyr	esc esp	AGA O'T	7CC	leu	CAC hts 150)	107	ACC.	AN I	TTC	CE T	AGC SRT	gi y	AAG lys	CYS	TCA ser (160)	gly
GTA :	OCG ele	STS val	TET	TCT SOF	ACE thr	TAC	Q1	TCC 170)	thr	MAC MAC	CAC his	GA T esp	TAC Lyr	ACC thr	ATT 11e	TGG trp		OCC pro (186)	glu
AAT (	CC G	#CA #73	CTA 1eu	gga gly	ATG	TET SOF	Cys	GAC MP 190)	110	111 <b>***</b>	ACC thr	AAT ASR	AGT SOT	ACA ATŞ	gi y	AAG lys	arg	9CA a) a (200)	107
AAA I	ecc ply	AGT SO	GAG glu	ACT thr	tox cys	gi y	-	67A wel 210)	45.0	gAA glu	#CA #73	esc gly	CTA	tat Cyr	AAG 1ys	TCT SET	leu	MA 1 ys (220)	gly
QCA 1	iox Cyri	AAA lys	CTC law	MG lys	TTA leu	TET Cys	917	611 161 230)	ieu	95A 9 <sup>1</sup> 7	CTT low	AÇA 679	CTT low	ATG	GAT asp	gga gly	thr	15G trp (240)	wa i

GCG ATG CAA ACA TCA AAT GAA ACC AAA TGG TGC CCT CCC GAT CAG TTG GTG AAC ale met gin the see asn glu the jys trp cys pro pro asp gin leu val asn (250) CTG TEXT GGACTGGCCGTCCTTTCANCCATCCAAGTCCTGAAGATCAGCTCCCCCTTGGGGGGGTT

fragment with avian myeloblastosis virus (AMV) reverse transcriptase extended the primer to the 5' terminus of the glycoprotein mRNA. Products of the reaction were resolved on a denaturing gel and identified by autoradiography (Fig. 3). Apart from the labelled primer fragment (actual length 36 nucleotides), the major products were ~40 and 165 nucleotides long. The 40-nucleotide band presumably arose by filling in the HindIII site recreated by the annealing of PvuII-HindIII DNA strands, while the 165-nucleotide band resulted from the extension of the primer which was hybridized to glycoprotein mRNA. As the PvuII site is located 129 bp from the 5' terminus of the cloned glycoprotein sequence (excluding the dG-dC tail of 24 bp), our results indicate that the cloned glycoprotein sequence is missing only ~35 nucleotides from the 5' terminus of the glycoprotein mRNA.

The complete nucleotide sequence of the glycoprotein cDNA (see Fig. 4) was determined by the Maxam and Gilbert technique<sup>10</sup> using both 5'- and 3'-labelled restriction fragments derived from both strands defined by the sites indicated in Fig. 2. From the nucleotide sequence, a polypeptide 524 amino acids long was deduced, beginning with an initiation codon—ATGlocated 8-10 nucleotides from the 5' end of the clone. The other two reading frames contained numerous stop codons, such that the longest polypeptide which can be derived from them is <50 amino acids long.

The first six amino acid residues of the mature glycoprotein, NH<sub>2</sub>-Lys-Phe-Pro-Ile-Tyr-Thr-, have been identified by direct amino acid sequence analysis of purified rabies virus (ERA strain) glycoprotein, as well as the C-terminal sequence Thr-Arg-Leu (C.-Y. Lai and B. Dietzschold, unpublished results). These sequences are located in the deduced sequence 20-25 and 522-524 residues from the putative initiating methionine. The initial 19 amino acids that precede the N-terminal lysine of the glycoprotein presumably represent a signal peptide as they are predominantly hydrophobic. An uninterrupted hydrophobic domain of 22 amino acids, bounded by lysine 439 (numbered from the N-terminal lysine) and two arginines at positions 462 and 463 near the carboxy-terminal region of the predicted polypeptide, is similar to the proposed transmembrane segment of Semliki Forest virus (SFV) glycoprotein<sup>11</sup>. A relatively long sequence of 44 charged and uncharged residues extends from the presumptive transmembrane domain to the C-terminal Leu 505 (compare with the viral glycoproteins of  $SFV^{11}$  and influenza virus<sup>12</sup>). This apparent hydrophilic cytoplasmic domain of the rabies glycoprotein may provide a site for strong interaction with the viral matrix  $(M_2)$  and nucleocapsid proteins.

The deduced amino acid sequence contains four carbohydrate acceptor sites as defined by the sequence Asn-X-Ser and Asn-X-Thr; three of these are located on the N-terminal side of the proposed transmembrane segment. Three carbohydrate chains have been found on the rabies virus glycoprotein of the ERA strain<sup>13</sup>, which agrees with the predicted number of carbohydrate attachment sites excluding Asp 465 within the putative cytoplasmic domain.

The absence of the sequence AAUAAA, present in eukaryotic polyadenylated mRNA14, in the 3'-noncoding region of rabies G mRNA and vesicular stomatitis virus mRNAs15 presumably reflects the role of virus-associated proteins in polyadenylation of these mRNAs<sup>16</sup> in place of host enzymes.

The cloned cDNA of rabies glycoprotein mRNA was readily identified and distinguished from nucleocapsid protein cDNA clones by (1) size selection of the poly(A) RNA identified by its ability to direct synthesis of glycoprotein in microinjected oocytes<sup>3</sup>; (2) hybridization with labelled purified rabies virion RNA; and (3) hybridization with glycoprotein mRNA as indicated by the correspondence of the profiles of hybridized RNA and RNA with translational capacity for glycoprotein. The accuracy of the cloned glycoprotein cDNA sequence was confirmed by the agreement between the predicted amino acid sequence and that determined by direct N-terminal and Cterminal amino acid analysis.

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# Physicochemical and antigenic properties of synthetic fragments of human leukocyte interferon

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Although interferons (IFNs) were originally described as proteins conferring antiviral resistance to eukaryotic cells<sup>1</sup>, various additional biological effects have since been attributed to them, including inhibition of cell proliferation and modulation of the immune response2. More recently it was recognized that interferons from different sources have similar amino acid sequences, suggesting their evolutionary relationship. When the sequences of human interferons of the  $\alpha$  and  $\beta$  type were aligned to give maximum homology, a largely conserved region was found near the carboxyl terminus of the molecules<sup>3,4</sup>. The homology of human leukocyte IFN-\alpha\_1 and human fibroblast interferon between positions 111 and 166, for instance, was 40%. As conservation of segments of polypeptide chains during evolution may indicate their functional importance<sup>5</sup>, we synthesized three carboxyl-terminal fragments of human IFN- $\alpha_1$  (ref. 6) ranging in size from 33 to 96 amino acid residues. If the folding of these fragments was similar to that of the corresponding segments in the intact protein, it was conceivable that they had biological activity and that antibodies raised against the synthetic fragments cross-reacted with natural interferon. Such antibodies could be used for the affinity purification of human interferon produced in bacteria and for radioimmunoassays. As we show here, none of the fragments had antiviral activity. A mouse monoclonal antibody prepared against a 56-residue fragment also bound intact IFN- $\alpha_1$  and IFN- $\alpha_2$  without neutralizing their antiviral effect. Thus, this antibody, directed against a largely conserved region, may show substantial cross-reactivity within the interferon protein family.

Three carboxyl-terminal fragments of human IFN- $\alpha_1$  (ref. 6), comprising residues 134-166, 111-166 and 71-166, were synthesized by the solid-phase method<sup>7-9</sup>. Experimental details are given for the 56-residue fragment because antibodies have previously been raised only against an extensively purified sample of this polypeptide. The sequence of the 56-residue fragment is shown in Fig. 1.

After deprotection and cleavage of the synthetic product from the solid support, the sulphydryl group of Cys 139 was blocked

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by reaction with iodoacetamide. In natural IFN- $\alpha_1$  this cysteine residue may form a disulphide bond with Cys 29, analogous to IFN- $\alpha A^{10}$ . The carboxamidomethylated fragment was purified on Sephadex G-50 (Fig. 2) and silica gel 60 and had the following amino acid composition (values expected are in parentheses): CM-Cys 1.0 (1), Asp 3.0 (3), Thr 2.9 (3), Ser 4.5 (5), Glu 5.7 (6), Pro 1.0 (1), Ala 4.1 (4), Val 2.7 (3), Met 2.0 (2), Ile 3.0 (3), Leu 8.0 (8), Tyr 2.8 (3), Phe 1.0 (1), Lys 5.0 (5), Trp 0.9 (1), Arg 6.7 (7). The synthetic 56-residue fragment of IFN- $\alpha_1$  was homogeneous on TLC in n-butanol/acetic acid/pyridine/water ( $R_F = 0.74$ ) and on thin-layer electrophoresis in 2 M HCOOH, 2 M urea at pH 2.0. Its aromatic region circular dichroism (CD) spectrum and fluorescence emission spectrum were typical of tryptophan-containing polypeptides.

The most striking physicochemical property of the 56-residue interferon fragment was its low solubility above pH7. Interferon 111-166 has a net charge of +6 whereas the entire IFN- $\alpha_1$ molecule has a net charge of -3. The natural protein contains 17 aromatic amino acid residues whereas the synthetic fragment contains 5, including 3 of the 4 tyrosines and 1 of the 2 tryptophans. Twenty-six of the 56 residues of the synthetic fragment are hydrophobic, with a calculated hydrophobicity index<sup>11</sup> of 31.9, lower than that of insulin (35.7), a protein of similar size. This suggests that the solubility properties of the interferon fragment cannot be explained by its amino acid composition.

The structure of synthetic interferon 111–166 in solution was investigated using CD. Analysis of the far-UV spectrum of a 0.1% solution of the synthetic fragment in 0.01 M phosphate, 0.09 M NaCl, pH 5.0, yielded values of 24 and 36% for  $\alpha$ -helix and  $\beta$ -sheet content respectively, using the method of Provencher and Glöckner<sup>12</sup>, in contrast to the secondary structure predicted for the corresponding region of IFN- $\alpha_1$  (65%  $\alpha$ -helix and 20%  $\beta$ -sheet)<sup>13</sup>. Interferon 111-166 was stable to urea denaturation up to a denaturant concentration of ~4 M but was unfolded by 8 M urea. The CD spectrum indicated that in the latter condition the molecule approximates a random coil. This process was almost fully reversible (>90%), the native CD spectrum being restored by diluting out the denaturant. This suggests that the fragment is capable of considerable selforganization, producing a structure that, in the light of the immunological results, is probably close to that in the intact interferon molecules.

Polyclonal antisera raised in rabbits or mice against the synthetic 56-residue fragment did not neutralize the antiviral activity of human leukocyte interferon. However, they seemed to bind to interferon, as binding to the fragment (assayed in a solid-phase radioimmunoassay as described in Table 1 legend) was competitively inhibited by Escherichia coli-derived leukocyte IFN- $\alpha_1$  or  $-\alpha_2$ , interferon from human buffy coat lymphocytes and virus-induced mouse interferon, but not by unrelated proteins (data not shown). This suggested that monoclonal

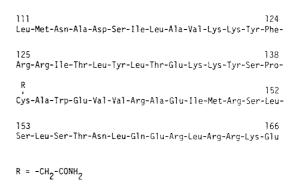


Fig. 1 Amino acid sequence of the synthetic carboxyl terminal 56-residue fragment of IFN-α<sub>1</sub> based on the nucleotide sequence of cloned cDNA<sup>6</sup>

**Table 1** Binding of monoclonal antibodies directed against the carboxyl-terminal 56-residue fragment of human leukocyte IFN- $\alpha_1$  to intact IFN- $\alpha_1$  and  $-\alpha_2$  and to various synthetic IFN- $\alpha_1$  fragments

			······································				
		125	I-labelled Staphyle	ococcus aureus prote	ein (c.p.m.) bound t	o plates coated with	
Clone	Ig subclass	Ovalbumin	IFN- $\alpha_1$	IFN-α <sub>2</sub>		ment corresponding	
			-	_	71-166	111-166	134-160
1/5	IgG3/κ	60	106	33	129	1,050	57
1/7	IgG2a/κ	111	153	71	598	1,201	1,069
II/3	IgG1/ĸ	164	169	164	661	1,085	524
II/11	IgG1/κ	166	177	155	725	1,230	653
II/14	IgG1/ĸ	90	181	55	743	1,067	987
II/20	IgG2a/ĸ	119	140	66	145	1,233	83
III/17	IgG3/ĸ	71	125	71	151	1,059	107
111/21	IgG1/ĸ	88	1.090	1.026	1.087	1,004	1.102
VII/8	IgG3/ĸ	82	242	200	463	1,135	407
XXI/5	IgG1/κ	156	163	84	563	1,064	866
XXIV/11	IgG2a/ĸ	94	187	87	673	1,271	1,264

The synthetic human IFN- $\alpha_1$  111-166 was intraperitoneally injected into three BALB/c mice as 50  $\mu$ g of protein mixed with complete Freund's adjuvant. A first booster injection with the same dose of antigen in incomplete Freund's adjuvant was given after 3 weeks. A second booster, after another interval of 4 weeks, consisted of 50  $\mu$ g of protein per animal injected intraperitoneally with incomplete adjuvant and 50  $\mu$ g of protein per animal dissolved in phosphate-buffered saline (PBS) and given intravenously. After 3 days, the spleen cells of the three animals were pooled and  $1.5 \times 10^8$  cells were fused to  $0.75 \times 10^8$  cells of the mouse myeloma cell line Fo using polyethylene glycol of molecular weight 4,000 as the fusing agent. Hybrid cells were selected for growth in hypoxanthine/aminopterin/thymidine medium. Supernatant fluids of cell cultures were screened for the presence of specifically binding antibodies in a solid phase radioimmunoassay. The 56-residue interferon fragment was dissolved in PBS at a concentration of 5  $\mu$ g ml<sup>-1</sup> and was adsorbed to polyvinylchloride microtitre plates. Additional protein binding sites were blocked by a 5-h exposure to 3% solution of ovalbumin. The supernatant fluids were exposed to the wells of microtitre plates for 4 h at room temperature. The presence of fragment-binding immunoglobulins was detected by assaying the radioactivity bound to the wells exposed to a rabbit anti-mouse immunoglobulin serum together with radiolabelled *S. aureus* protein. Cells of initially positive cultures were cloned twice by a limiting dilution technique and injected into female BALB/c mice. Ascitic fluids, diluted 1:1,000, were tested as described above. The 33- and 96-residue fragments of interferon were adsorbed to plates as described for the 56-residue peptide. *E. coli-derived* human leukocyte IFN- $\alpha_1$  and - $\alpha_2$ , each at a concentration of 10  $\mu$ g ml<sup>-1</sup> in PBS and of 10% purity, were similarly adsorbed to the plates. Each value shown represents the mean counts per min from th

antibodies<sup>14</sup> prepared against the 56-residue fragment recognize antigenic sites on the corresponding segments of intact interferon molecules.

Three BALB/c mice were immunized against the 56-residue fragment, their spleen cells pooled and fused to cells of the mouse myeloma line Fo (ref. 15), and hybrid cells selected for production of fragment-binding antibodies as described in Table 1 legend. Twenty-eight hybrid cell cultures obtained from the fusion of  $1.5 \times 10^8$  spleen cells with  $0.75 \times 10^8$  myeloma cells were found to produce proteins that bound to the fragment. Sixteen of the originally positive cultures were cloned twice; of these clones, five produced proteins that cross-reacted with the unrelated antigen ovalbumin, the remaining 11 synthesizing specific antibodies of subclasses IgG1, IgG2a and IgG3, all containing  $\kappa$  light chains.

Further analysis of the binding properties of these specific antibodies in a radioimmunoassay using the synthetic carboxylterminal 96- and 33-residue fragment and bacterially produced IFN- $\alpha_1$  and - $\alpha_2$  (Table 1) suggested that the hybridoma antibodies fell into three classes: antibodies of clone III/21 bound to all fragments and both interferons; antibodies of clones I/7, II/3, II/11, II/14, VII/8, XXI/5 and XXIV/11 bound to various degrees to all fragments but not to the interferons; the products of clones I/5, II/20 and III/17 bound exclusively to the 56-residue fragment. Thus, at least three different epitopes are recognized on the 56-residue fragment, one of which is accessible to a distinct monoclonal antibody also on natural interferon.

Ascitic fluid from mice bearing tumours of clone III/21 was tested for neutralization of the antiviral effect of IFN- $\alpha_1$  and  $-\alpha_2$  on three different target cells (human larynx carcinoma line CCL 23, bovine kidney cells and mouse L-cells) on which both interferons show cross-reactivity<sup>16</sup>, using Mengo virus, vesicular stomatitis virus or encephalomyocarditis virus as challenge. No reduction of the interferon titres was found after preincubation with ascitic fluid from clone III/21 diluted 1:20, whereas at a dilution of 1:10 the ascitic fluid neutralized a maximum of 3 IFN units and at 1:5 it neutralized a maximum of 9 IFN units. This minor reduction of the interferon titres, however, may have been partly caused by ascitic fluid components other than the monoclonal antibody. The fact that the antibodies raised against the synthetic 56-residue fragment essentially did not neutralize the interferon activity was compatible with the observation that

none of the synthetic fragments inhibited viral growth in the cell lines mentioned above.

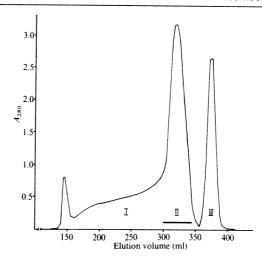
Human leukocyte interferon extracted from bacteria is largely contaminated by prokaryotic proteins. As mouse hybridomas secreting monoclonal antibodies to interferon represent a rich source of material for the preparation of affinity columns, we investigated the efficacy of affinity chromatography for the purification of human interferon produced in bacteria.

Mice were injected with cells of clone III/21 and their ascitic fluids, binding to IFN- $\alpha_2$ -coated plates with a titre of  $10^{-5}$ pooled. The IgG fraction was isolated and coupled to CNBractivated Sepharose 4B. A column with a capacity of  $2 \times 10^7$ reference units of IFN- $\alpha$  per ml of gel was loaded with partially purified IFN-α<sub>2</sub> (E. coli) (Fig. 3, slot 2) which had a specific activity of  $3 \times 10^6$  units per mg of protein. The column was eluted with phosphate-buffered saline (PBS, pH 7.2), followed by McIlvaine's buffer (pH 2.6). At neutral pH most of the interferon was retained. The material eluting at pH 2.6 was enriched in interferon (4.8 × 10<sup>7</sup> units per mg of protein) and low molecular weight (M<sub>r</sub>) proteins and was almost free of high molecular weight proteins as shown by SDS-polyacrylamide gel electrophoresis (Fig. 3, slot 3). Re-chromatography of this material in identical conditions gave purified interferon with a specific activity of 8.8×10<sup>7</sup> units per mg of protein. Gel electrophoresis of the fraction eluting at pH 2.6 showed a very strong interferon band corresponding to a M, of 19,400, a minor band at  $M_r$  17,800, and a very weak band at  $M_r$  13,000 (Fig. 3, slot 4).

The synthetic carboxyl-terminal 56-residue fragment of human IFN- $\alpha_1$  was not homogeneous: although its amino acid composition as well as chromatographic and electrophoretic purity were satisfactory (the good recovery of tryptophan is particularly noticeable), minor deviations from the target sequence caused mainly by deletions at various stages of the synthesis could not be excluded. Further purification of the 56-residue fragment will involve affinity chromatography on an antibody column and HPLC. However, the CD measurements indicated that the synthetic IFN 111-166 had a considerable amount of stable structure.

Synthetic fragments of natural proteins may be suitable for raising antisera that cross-react with the parent molecules 17,18. The present study describes the first production of a monoclonal antibody against a synthetic polypeptide, the linear carboxyl-

Fig. 2 Fractionation of the carboxamidomethylated carboxyl-terminal 56residue fragment of human IFN-α<sub>1</sub> (105 mg) on a Sephadex G-50 column (97.5 × 2.4 cm) in 0.05 M CH<sub>3</sub>COOH. I, disulphide dimer and aggregates; II, monomeric carboxamidomethylated 56-residue fragment (the bar indicates the elution range of the fraction that was re-chromatographed); III, excess iodoacetamide. The carboxamidomethylated 56-residue fragment was obtained as follows<sup>7-9</sup>. Tertiary butyloxycarbonyl (Boc) amino acids were coupled in a threefold molar excess based on the amount of carboxyl terminal glutamic acid esterified to the resin. Each coupling reaction was repeated with a twofold molar excess of the Boc amino acid. The side chains of methionine and tryptophan were unprotected. To prevent their alkylation or oxidative cleavage during the acid deprotection step, 10% anisole and 2%  $\beta$ -mercaptoethanol were present in the deblocking mixture (40% trifluoroacetic acid in CH<sub>2</sub>Cl<sub>2</sub>) after addition to the peptide resin of Boc-Met and Boc-Trp, respectively. At the end of the synthesis the yield of protected 56-residue peptide resin was 85.8%. The synthetic polypeptide was deprotected and cleaved from the resin by anhydrous HF in the presence of anisole (sixfold molar excess per protecting group) and free tryptophan (twofold molar excess per tryptophan residue) at 0 °C for 30 min and then extracted by 1 M CH<sub>3</sub>COOH. After lyophilization it was dissolved in 0.05 M NH4HCO3, 8 M urea and dialysed against 0.05 M NH4HCO3. Decreasing urea concentration caused the synthetic 56-residue polypeptide to pre-



cipitate, and hyphilization gave the crude interferon fragment. The yield of the cleavage step was 82.9%. The synthetic fragment was treated with a 100-fold molar excess of  $\beta$ -mercaptoethanol in 5 M urea at pH 4 overnight and the reduced protein was isolated by gel filtration on Bio-Gel P-2 in 0.1 M CH<sub>3</sub>COOH. To the pooled protein fractions 0.05 M NH<sub>4</sub>HCO<sub>3</sub> was added to give a final protein concentration of 1mg mi<sup>-1</sup>. The sulphydryl group of Cys 139 was blocked by reaction with a 10-fold molar excess of iodoacetamide at pH 7 and the product was fractionated on Sephadex G-50 as shown above. After re-chromatography the carboxamidomethylated 56-residue fragment was obtained in 52.5% yield based on the amount applied to the first column. It was purified further by adsorption chromatography on silica gel 60 using n-butanol, acetic acid, pyridine and water (15:3:10:12) as eluant

terminal 56-residue fragment of human IFN- $\alpha_1$ . The crossreactivity of antibodies of clone III/21 with the synthetic interferon fragments 134-166 and 71-166 and with complete human IFN- $\alpha_1$  and  $-\alpha_2$  (derived from E. coli) suggested that there was a common antigenic site of very similar fold that was (largely) located between residues 134 and 166 of the interferon sequence, and accessible on the natural interferon molecules. Monoclonal antibodies that bound the synthetic fragments only, probably recognized structures that were either absent or not accessible on natural interferons.

A small library of monoclonal antibodies against leukocyte interferons already exists. A monoclonal antibody described by Secher and Burke<sup>19</sup> was raised against human leukocyte interferon and selected for its capacity to neutralize the antiviral effect of this protein. It did not cross-react with human fibroblast or mouse interferon<sup>20</sup>. As the antibodies prepared to the synthetic carboxyl terminal 56-residue fragment of IFN- $\alpha_1$  were essentially non-neutralizing, the binding site of the monoclonal antibody of Secher and Burke was either located between residues 1 and 110 or was constituted by amino acid residues scattered over a large part of the interferon sequence including

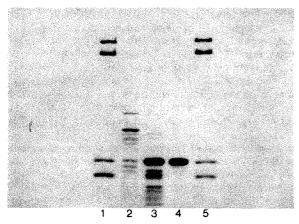


Fig. 3 Gel electrophoretic analysis of E. coli-derived human IFN- $\alpha_2$  before and after affinity chromatography on a column containing a monoclonal antibody prepared against the carboxyl terminal 56-residue fragment of human IFN-α<sub>1</sub>. Gel, 0.1% SDS-17% polyacrylamide; 25 μg of protein were applied per slot. Staining was with Coomassie brilliant blue. 1, 5, Protein markers (from top to bottom: bovine serum albumin, catalase,  $\beta$ -lactoglobulin, cytochrome c). 2, Partially purified interferon applied to the antibody column. 3, Material eluted with McIlvaine's buffer, pH 2.6; after neutralization it was re-chromatographed on the antibody column. 4, Rechromatographed material eluting at pH 2.6; the strong band at molecular weight 19.400 is interferon.

the region from 111 to 166. It was also possible that a neutralizing site at the carboxyl terminus of human leukocyte interferon was not present on the synthetic fragment because of conformational differences. Another monoclonal IgG specific for human leukocyte interferon was obtained by Montagnier et al.21, while Staehelin and co-workers22 prepared 13 monoclonal antibodies to human leukocyte interferon which gave distinct binding patterns with purified interferon species and of which were non-neutralizing. The monoclonal antibody described here bound (at least) human IFN- $\alpha_1$  and - $\alpha_2$  derived from E. coli and was used to prepare an affinity column from which purified human IFN- $\alpha_2$  (E. coli) with a specific activity of ~10<sup>8</sup> units per mg of protein was isolated. Ultimately, a whole library of monoclonal antibodies may be available to map the antibody and receptor binding sites of the various interferons.

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# Carrier-dependent and carrierindependent transport of anti-cancer alkylating agents

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While it is understandable that proliferating cells should be more sensitive than resting cells to antimetabolites—drugs that derange DNA synthesis—It is not so obvious that other anticancer drugs, in particular the alkylating agents, should act in the same way. The greater sensitivity of proliferating cells to such agents is not confined to malignant tissues: normal haematopoletic cells show increased susceptibility after recruitment into cell division4. Moreover, the various alkylating agents have different effects on proliferating nonmalignant cells; in normal bone marrow, recovery is more rapid and greater after treatment with some drugs (for example, cyclophosphamide and nitrogen mustard) than others (such as nitrosourens and mitomycin C). We now provide evidence in support of the hypothesis that there are two distinct groups of alkylating agent: those whose uptake into the cell depends on a membrane transport mechanism (that is, carrier-dependent) and those whose uptake is not so dependent (carrier-independent).

An assay which uses the mitogen phytohaemagglutinin (PHA) to induce proliferation in tissue culture of human peripheral T lymphocytes<sup>7-9</sup> was developed for these experiments<sup>10</sup>. It can be seen (Fig. 1) that <sup>3</sup>H-thymidine uptake was very low before PHA stimulation but increased steadily (as measured by continuous label incorporation) in the 18 h after its addition. Therefore, in this assay, unstimulated human peripheral T lymphocytes are considered a 'resting' cell population and after 18 h of exposure to PHA they are considered to be actively proliferating. To test the effects of alkylating agents on these two populations of cells, various drug concentrations were added either 60 min before or 18 h after PHA stimulation. An initial experiment used melphalan (L-phenylalanine mustard), which has recently been shown to be actively taken up by an amino acid transport system in both rodent cells and human lymphocytes 11-13. Figure 1 shows the effect of a 60-min exposure to melphalan at various periods (between 0 and 26 h) after PHA stimulation. Initially, melphalan caused only ~30% death of resting human peripheral Tlymphocytes, but its toxicity steadily increased as PHA-induced proliferation increased, resulting in a maximum of ~90% death 8 h after addition of PHA.

We then determined quantitatively the cytotoxicity of various alkylating agents and X rays, using a series of increasing drug concentrations to obtain reasonably complete (2-3 log death) survival curves. In each case the cells were exposed to the agent for 60 min (an arbitrary exposure time) either at time 0 (resting cells just before PHA exposure, solid circles in Figs 2 and 3) or 18 h after PHA-induced proliferation (cycling cells, open circles in Figs 2 and 3). We obtained two clearly distinguished survival patterns. The first of these we term carrier-dependent (see Fig. 2). Carrier-dependent toxicity is characterized by dose-dependent cell death but with a substantially greater shoulder on the survival curve of resting cells. The shoulder is reduced or eliminated when the cells are exposed to the carrier-dependent agent after induction of cell cycling. Melphalan, nitrogen mustard and cis-dichlorodiammineplatinum (CDDP) all gave this pattern. These three agents are highly water-soluble and two are known to be transported into cells by membrane carriers (melphalan by amino acid carriers 11-13 and nitrogen mustard by a choline carrier<sup>14,15</sup>). We have also found that amino acids can protect cells against CDDP, suggesting that the latter is probably also taken up by an amino acid transport mechanism<sup>16</sup>, which may explain its nephrotoxicity<sup>17</sup>. A fourth water-soluble alkylating agent, phosphoramide mustard (the active form of cyclophosphamide), proved almost inert (at concentrations up to 100 µg ml<sup>-1</sup>) against both resting and cycling human T cells, a feature which may explain its well-documented differential sparing of T cells in vivo<sup>18</sup>.

The second survival pattern, which we term carrier-independent toxicity, is shown in Fig. 3. All the carrier-independent

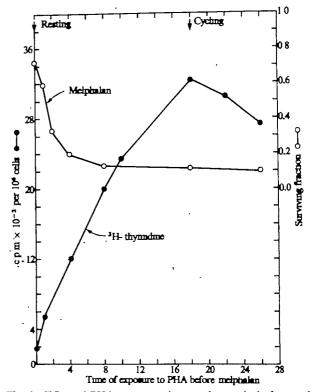
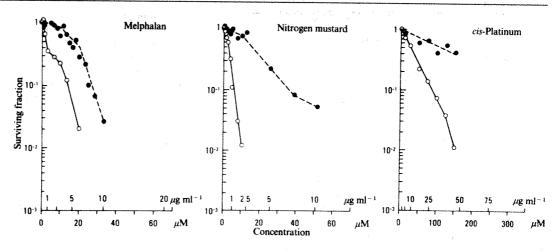


Fig. 1 Effect of PHA exposure time on the survival of normal peripheral T lymphocytes exposed to melphalan -1) for 1 h (O) and on the incorporation of <sup>3</sup>H-thymidine human (4 µg ml" continuous label (.). The assay procedure for determining drug cytotoxicity of normal human T lymphocytes was as follows. Peripheral blood from a healthy adult was collected with 50 U ml heparin and the mononuclear cells were isolated by Ficoll-Paque (Pharmacia) gradient centrifugation. Cells from the interphase containing >95% lymphocytes were washed three times in Hank's balanced salt solution (HBSS; Gibco) and resuspended at 106 cells ml<sup>-1</sup> in McCoy's 5a medium containing 15% heat-inactivated fetal bovine serum (FBS; Gibco). Aliquots (1 ml) of the cell suspension were seeded into 16 × 125 mm tubes (Falcon 3033); replicate tubes were used for determining cell survival or DNA synthesis. PHA (HA15 Wellcome) was freshly prepared at 2% in complete medium and 1 ml added to each tube at time 0; one set of tubes (♠) contained [Me-³H]thymidine (43 Ci mmol⁻¹, 10 μCi ml⁻¹; Amersham). At the times indicated, one tube from each set was removed and DNA synthesis determined (1). The other tube (0) was exposed to melphalan (NSC 8806, freshly prepared at 4 µg final concentration in complete medium) for 1 h, followed by washes and re-exposure to PHA for the remaining time necessary to complete an 18-h PHA exposure for colony formation. The cells were then washed in HBSS and syringe-resuspended in 2 ml complete medium to disaggregate the cells. To each tube were added 1 ml of 1% PHA in complete medium and 2 ml 2.6% methylcellulose in complete medium. Cells were plated in 35-mm dishes (Falcon) to give a final concentration per plate of  $5\times10^5$ cells in 0.2% PHA, 1.0% methylcellulose. PHA-induced colonics containing >50 cells were counted with an inverted microscope at day 7 Plating efficiencies for non-treated controls in both the resting and cycling sets were consistently ~0.1%. Surviving fractions were determined by assigning a value of 1.0 to the untreated controls. DNA synthesis was determined by the method of Evans and Norman  $^{52}$ . Briefly, the labelled cells were washed three times in cold PBS containing  $100~\mu \mathrm{g}~\mathrm{m}\mathrm{l}^{-1}$  thymidine (Sigma) and resuspended in 10 ml cold PBS. Cell number was determined and cells placed on Millipore GS 0.22-µm filters. Filters were washed with 5% trichloroacetic acid, then 95% ethanol, and air-dried overnight and placed in viels with 10 ml scintillation fluid.

Fig. 2 Effect of carrierdependent agents on the survival of normal human lymphocytes before (
) and after PHA stimulation for 18 h (O). The overall assay procedure is outlined in Fig. 1 legend. The two different time points for the 60-min exposure are indicated by arrows in Fig. 1. All drugs were freshly prepared before use. Melphalan (NSC 8806) was dissolved 10 mg ml (9:1). ethanol/HCl Nitrogen mustard (Mustargen) was obtained as a 1 mg ml<sup>-1</sup> solution in solution in



PBS; cis-platinum (Platinol; Bristol) was obtained as 10 mg lyophilized sample and reconstituted with sterile double distilled water. All dilutions were made in complete media.

drugs gave identical survival curves for resting and cycling cells and all are highly lipid-soluble. Those drugs which showed this pattern (Fig. 3) include the nitrosureas 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and methyl-CCNU [1-(2-chloroethyl)-3-trans-(4-methyl-cyclohexyl)-1-nitrosourea] (plus CCNU, data not shown), procarbazine, busulfan (data not shown), mitomycin C, an amphipathic agent freely soluble in both water and lipids<sup>19</sup>, and ACNU [1-(4-amino-2-methyl-pyrimidin-5-yl)-methyl-3-(2-chloroethyl)-3-(nitrosourea)], an amphipathic nitrosourea with delayed marrow toxicity<sup>20</sup>. These results

suggest that the extent of lipid solubility (which would override a carrier mechanism) may dictate the type of cell survival pattern. X rays (whose entry into cells is independent of any 'carrier') also showed a carrier-independent pattern. As these nitrosoureas<sup>21,22</sup> and mitomycin C<sup>23</sup> are bifunctional alkylating agents and cross-link DNA in a manner similar to that of the classical mustards, it seems unlikely that it is the nature of the molecular damage which results in the different survival patterns. Repair of DNA damage in resting cells (that is, before cycling) also cannot explain the relative resistance of resting cells

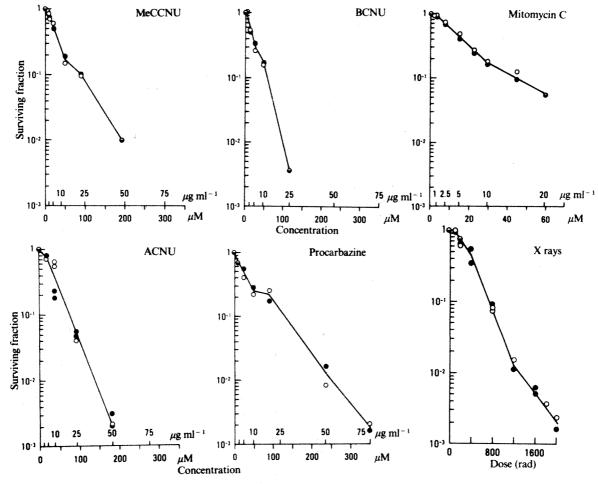


Fig. 3 Effect of carrier-independent agents on the survival of normal human lymphocytes before (●) and after PHA stimulation for 18 h (○). The assay procedure is outlined in Fig. 1 legend. All drugs were freshly prepared before use. Methyl-CCNU (MeCCNU; NSC 95441), BCNU (NSC 409962), ACNU (NSC D245382) and procarbazine (NSC 77213) were dissolved at 1 mg ml<sup>-1</sup> in absolute ethanol. Mitomycin C (Mutamycin; Bristol) was obtained as 5 mg in sterile distilled water. All dilutions were made in complete media. X-ray exposure was at room temperature in air on a Picker orthovoltage 280 keV machine, using an HVL 1.0 mm copper filter with an output of 150 rad min<sup>-1</sup>.

to carrier-dependent drugs, as the carrier-independent alkylating agents would be expected to show the same evidence of cellular repair. The feature which dictates the shape of the survival curve and governs the (relative) independence/dependence of toxicity on the cell's proliferative state seems to be the drug's ability to penetrate the cell by simple diffusion into cellular lipids and lipoproteins<sup>24,25</sup>.

We believe these two patterns are reflected in the two recovery patterns shown by bone marrow after exposure to alkylating agents. When delivered as a bolus, the recovery of marrow from carrier-dependent agents is usually fairly rapid such that cyclical therapy, can be repeated about every 21 days 5.26-28. The equivalent administration of a lipid-soluble nitrosourea<sup>20,29</sup>, mitomycin C<sup>30,31</sup>, or X rays<sup>28</sup> is followed by a considerably prolonged marrow recovery period of 4-6 weeks. In the rat this delayed recovery pattern has recently been shown (for CCNU) to relate to marrow stem-cell killing independent of the proliferative state of the pluripotential marrow stem cells<sup>32</sup>. Clinically, all the carrier-independent agents (those cited in Fig. 3 legend, plus CCNU and busulfan) are associated with delayed marrow recovery and also with cumulative marrow damage, that is, a steady irreversible depletion of marrow reserve with progressive dosage<sup>5,20,33-35</sup>. On the other hand, the carrier-dependent agents produce relatively less marrow damage and show much more rapid marrow recovery<sup>6,28</sup>. The only apparent exception to this rule is melphalan<sup>31,36</sup>; we believe this discrepancy is due to the prolonged administration schedule that has been used for melphalan in man.

This binary model may also be relevant to the means by which tumour cells develop resistance. It predicts that a significant source of resistance to carrier-dependent alkylating agents could stem from somatic mutations that yield cells whose membrane carriers are either fewer in number or have reduced drug affinity. A contribution to resistance by reduced transmembrane transport has recently been shown for melphalan<sup>13</sup>. As CDDP seems to be transported by a similar pathway<sup>16</sup>, its partial cross-resistance with melphalan<sup>37,38</sup> is perhaps expected.

For carrier-independent agents, cell uptake seems to be independent of membrane events and the existence of somatic mutants conferring significant resistance at the membrane level is unlikely. This suggests that 'resistance' to carrier-independent alkylating agents can stem only from intracellular sources, for example, by reduced intracellular activation of the agent (if such is needed), selection of cells with greater DNA repair mechanisms<sup>38–42</sup> or by nonspecific protective effects such as the selection of mutants with elevated thiol levels<sup>43</sup>.. The reduced tendency of carrier-independent drugs to induce resistance<sup>44</sup>, and their high activity in some rodent models 45, make carrierindependent agents superficially attractive. However, the data in Fig. 3 indicate that such drugs do not have any true differential cytotoxicity between resting and cycling normal stem cells. These properties reduce their overall clinical potential considerably as regimens exploiting the considerable recruitment potential of resting normal tissues are precluded.

These data suggest that the proliferation dependency of alkylating agent cytotoxicity stems from two unequal and disparate sources: (1) a major dependence, limited to carrierdependent drugs, on enhanced transport of the drug into the cell because it is, in part, a stereochemical analogue of a normal compound (for example, amino acid, choline) used in cell biosynthesis; (2) a minor dependence (shared by both carrierdependent and carrier-independent drugs) attributable to inherent sensitivity of replicating DNA to alkylating agents. We recently showed that the cytotoxicity exerted by alkylating agents seems to be a function of the number of DNA replicons that encounter unrepaired alkylated sites on the DNA. These data, together with recent studies 47 on the mechanism of recovery from CDDP damage of resting tissue culture cells suggest this to be the molecular basis for the enhanced sensitivity of cycling cells to both carrier-dependent and carrier-independent alkylating agents.

There are several implications of this model: (1) carrierdependent agents are likely to be more useful clinically than those that are carrier-independent, and new, useful carrierdependent alkylating agents can probably be synthesized that will exploit membrane carriers not available to present agents (for example, bases, sugars, other amino acids, polyamines); (2) the minimal dose of each carrier-dependent agent used in each treatment cycle should probably be at or just beyond the point where the differential effect of the agent against resting versus cycling cells is first maximized; (3) treatment schedules which include and serially alternate several carrier-dependent agents known to be lacking in cross-resistance are predicted to be most useful, as this would minimize the outgrowth of drug-resistant (membrane) mutants<sup>48,49</sup>; (4) the inclusion of carrier-independent agents in these schedules is likely to be harmful, except in the rare event where a specific advantage such as central nervous system penetration or hypoxic activation<sup>50</sup> exists, or where the net tumour volume is minimal and ('adjuvant') non-selective chemotherapy might be effective; (5) therapies designed to enhance proliferation-dependent carrier-dependent drug killing (for example, combinations of carrier-dependent alkylating agents and clinically tolerable methylated xanthines, for example see ref. 51) might also usefully be examined.

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# Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets

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Streptozotocin, which produces diabetes mellitus in experimental animals <sup>1-3</sup>, has been reported to reduce the level of NAD in pancreatic islets <sup>4,5</sup> and to inhibit islet synthesis of proinsulin <sup>6</sup>. The decrease in NAD is due to increased NAD degradation mediated by islet nuclear poly(ADP-ribose) synthetase <sup>7,8</sup>. Evidence suggests that poly(ADP-ribose) synthetase is activated when DNA is fragmented <sup>9-17</sup>. Here we describe that both streptozotocin and alloxan, which also produces experimental diabetes mellitus <sup>1,2</sup>, cause DNA strand breaks which stimulate nuclear poly(ADP-ribose) synthetase, thereby depleting intracellular NAD and inhibiting proinsulin synthesis in isolated pancreatic islets of rats.

Islets isolated from rat pancreas were incubated with streptozotocin or alloxan for 5-20 min in Krebs-Ringer's bicarbonate medium and velocity sedimentation of DNA from the islet cells was carried out in an alkaline sucrose gradient. DNA from islets incubated for 10 min without the diabetogenic agents was recovered as a single peak near the bottom of the gradient, the position at which undamaged DNA sediments (Fig. 1a, e). However, after only 5 min incubation with 2 mM streptozotocin or 1 mM alloxan, a considerable amount of DNA sedimented as a broad peak in the middle of the gradient with a concomitant decrease in undamaged DNA (Fig. 1b, f); after 10-20 min incubation, the DNA was almost completely fragmented (Fig. 1c, d, g, h). The effect of the two agents on islet DNA fragmentation was dose dependent. These results clearly indicate that streptozotocin and alloxan produce strand breaks in islet DNA.

DNA fragmentation in mammalian cells has been reported to activate nuclear poly(ADP-ribose) synthetase<sup>9-15</sup>, and there is direct evidence 16,17 that poly(ADP-ribose) synthetase purified from bovine thymus is activated only when the enzyme is bound to fragmented or nicked DNA. Therefore, we prepared a nuclear fraction from islets incubated in conditions causing breaks in islet DNA, and assayed poly(ADP-ribose) synthetase activity. As shown in Fig. 2a, b, both streptozotocin (2 mM) and alloxan (1 mM) induced a two- to threefold increase in islet poly(ADP-ribose) synthetase activity, with a peak at 10 min. Streptozotocin has also been shown to cause an approximately 1.2-3-fold increase in nuclear enzyme activity of Physarum polycephalum<sup>18</sup> and HeLa cells<sup>19</sup>; and as much as a 10-fold increase in enzyme activity has been observed in y-irradiated permeable cells<sup>20</sup>. The procedure used to isolate the nuclei is reported<sup>10,20</sup> to cause DNA strand breaks and to activate poly(ADP-ribose) synthetase. We therefore examined a DNA sedimentation profile of the islet nuclear fraction. We found that the isolation procedure per se caused some DNA breaks, but that nuclear DNA from islets incubated with alloxan or streptozotocin was fragmented to a greater degree than that induced by the nuclear isolation procedure alone (data not shown). This indicates that the low, two- to threefold increase in islet nuclear poly(ADP-ribose) synthetase activity was probably due to the high background of DNA breaks resulting from the isolation procedure.

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Cellular NAD content was reduced by either 2 mM streptozotocin or 1 mM alloxan within 20 min of incubation (Fig. 2c, d), and remained almost unaltered for 60 min. There was striking temporal correlation between the decrease in level of islet NAD and the increase in islet poly(ADP-ribose) synthetase activity. These results indicate that both alloxan and streptozotocin cause DNA strand breaks which result in an increase in poly(ADP-ribose) synthetase activity, thereby depleting islet NAD level. However, it has been suggested that alloxan may not be toxic to islets because it decreases the NAD level<sup>2.3</sup>.

Next, we examined the effect of inhibitors of poly(ADP-ribose) synthetase on streptozotocin- or alloxan-reduced islet NAD content. The inhibitors used were nicotinamide and picolinamide, which are potent inhibitors of islet nuclear poly(ADP-ribose) synthetase<sup>7</sup>. The results are shown in Table 1. Alloxan (1 mM) decreased islet NAD level to 18% of the control level (without alloxan). Nicotinamide (2 mM) or picolinamide (2 mM) completely abolished the alloxan-induced decrease in islet NAD level. As previously described<sup>5,7</sup>, the same was true for the decrease in islet NAD level induced by streptozotocin. Addition of nicotinamide or picolinamide alone produced only a slight increase in islet NAD level.

The ability of islets to synthesize proinsulin is considered a crucial marker for evaluation of diabetogenicity of alloxan and streptozotocin<sup>21,22</sup>. As shown in Table 2, 1 mM alloxan reduced proinsulin synthesis to 9% of the control level; nicotinamide (2 mM) or picolinamide (2 mM) reversed this decrease to 35% or 27% of the control value. The decrease in proinsulin synthesis induced by 0.5 mM alloxan (30% of the control) was almost completely abolished by nicotinamide or picolinamide. The decrease in proinsulin synthesis induced by streptozotocin (1 or 2 mM) was also significantly reversed by the addition of either nicotinamide or picolinamide, as described elsewhere<sup>7</sup>. In addition to these *in vitro* experiments with isolated rat islets, *in vivo* experiments on rats confirmed that nicotinamide and picolinamide prevent development of alloxan- as well as streptozotocin-induced diabetes (unpublished data).

This study demonstrates that streptozotocin and alloxan cause DNA strand breaks to stimulate nuclear poly(ADP-ribose) synthetase, thereby depleting intracellular NAD and inhibiting proinsulin synthesis. Inhibitors of islet nuclear poly(ADPribose) synthetase such as nicotinamide and picolinamide were found to reverse the reduction in NAD level and also the inhibition of proinsulin synthesis. These inhibitors may prevent the diabetogenic action of alloxan and streptozotocin by inhibiting NAD degradation through poly(ADP-ribose). Other species of poly(ADP-ribose) synthetase inhibitors, such as 3aminobenzamide, 3-nitrobenzamide and 3-methoxybenzamide, were found to protect against the depression of proinsulin synthesis induced by streptozotocin or alloxan (H.Y., A. Kawamura and H.O., in preparation). Methylxanthines may protect against the action of alloxan on islets23 in a similar way, because they also have been reported to inhibit poly(ADPribose) synthetase<sup>24,25</sup>

Thiols<sup>26</sup> and radical scavengers<sup>27</sup> can also protect against the diabetogenic action of alloxan. It has been suggested that hydroxyl radicals generated from alloxan mediate its diabetogenic action<sup>27-29</sup>. More recently, we observed that strand breaks in islet DNA by alloxan were abolished in the presence of superoxide dismutase (Y.U., H.Y. and H.O., in preparation). Thus it is likely that the free radicals generated from alloxan damage islet cell DNA, resulting in activation of poly(ADP-ribose) synthetase.

The increase in islet poly(ADP-ribose) synthetase activity induced by streptozotocin or alloxan resulted in a significant depletion of islet NAD content. This reduction in intracellular NAD to such a non-physiological level may severely affect islet cell functions, including proinsulin synthesis. In addition, certain viruses or physical factors such as  $\gamma$  rays, which are known to cause DNA strand breaks and to stimulate poly(ADP-ribose) synthetase<sup>11-15,30,31</sup>, may be expected to be aetiological agents of insulin-dependent diabetes<sup>8</sup>.

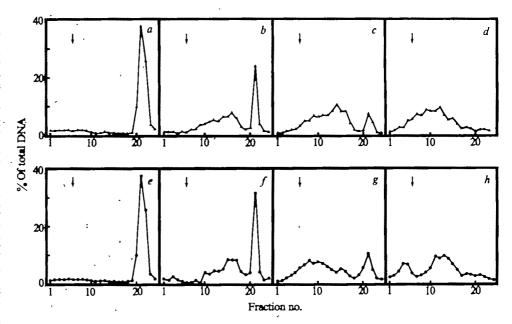
Table 1 NAD content of islets incubated in the presence of alloxan or streptozotocin with or without the addition of nicotinamide or picolinamide

Addition	Total NAD in PCA extract (pmol)	<sup>14</sup> C-NAD in PCA extract (c.p.m.×10 <sup>4</sup> )	% Recovery	Islet NAD content (pmol per islet)
None	351	4.16	93.2	2.84 (100)
Alloxan (1 mM)	141	4.37	97.9	0.52 (18)
Alloxan (1 mM) and nicotinamide (2 mM)	369	4.26	95.5	2.94 (104)
Alloxan (1 mM) and picolinamide (2 mM)	364	· 4.35	97.6	2.81 (99)
Streptozotocin (2 mM)	116	4.10	92.0	0.34 (12)
Streptozotocin (2 mM) and nicotinamide (2 mM)	365	4.25	95.4	2.91 (102)
Streptozotocin (2 mM) and picolinamide (2 mM)	380	4.40	98.6	2.93 (103)
Nicotinamide (2 mM)	395	4.29	96.1	3.19 (112)
Picolinamide (2 mM)	377	4.19	94.0	3.09 (109)

Batches of 100 islets were incubated at 37 °C for 20 mm and NAD content of the islets determined as described in Fig. 2 legend.  $^{14}$ C-NAD  $(4.46 \times 10^4 \text{ c.p.m.}, 92.1 \text{ pmol})$  was added to each sample before disruption of the islets, and the recovery of  $^{14}$ C-radioactivity in the final preparation used to correct the NAD value for overall recovery. Numbers in parentheses are islet NAD content as a percentage of NAD content in cells without any additions.

Fig. 1 Sedimentation profiles in an alkaline sucrose gradient of DNA from salets incubated with streptozotochi or alloxan. Pancreatic salets of Langerhans were iso-lated by the method of Okamoto et al. 22 from male Wistar rats weighing 200-250 g, which were fed ad libition. Batches of 100 islets were incubated for 5-20 min at 37 °C m 100 µl Krebs-Ringer's bicarbonate medium, pH 7.4, supplemented with 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium gluta-mate, 2 mg ml<sup>-1</sup> bovine serum albumin (BSA) and 2.8 mM glucose in an atmosphere of 95% O<sub>2</sub>/5% CO<sub>2</sub> (refs 21, 32) Streptozotocin (Upjohn) and allowen monohydrate (Wako Pure Chemical Industries) were dissolved in distilled water just before addition because of their relatively short half lives 1.2. After incubation, islets were suspended in  $50 \, \mu l$  of cold 0.9% saline and immediately layered over 0.5 ml lysis solution (1 M NaOH, 0.01 M EDTA, 1% (v/v) Triton X-100) that had just been layered over 14.8 ml of a 5-20% (w/v) linear sucrose gradient containing 0.3 M NaOH, 0.7 M NaCl and 0.01 M EDTA. On the bottom of each gradient was a 1 ml 80% (w/v) sucrose shelf. The

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loaded gradients were placed in the dark at room temperature for 30 min, then centrifuged at 26,000 r.p.m. at 20 °C for 200 min in a Beckman SW27.1 rotor. After centrifugation, fractions of 33 drops were collected from the gradient. DNA in each fraction was precipitated by adding 2 ml of 20% cold trichloroacetic acid (TCA) with 200 µg BSA as carrier. The precipitate was washed 3 times with cold TCA, once with cold 0.1 M potassium acetate m ethanol, there with ethanol, then assayed for DNA content by a finorimetric method<sup>33</sup>. Each point represents the percentage of total DNA recovered; recovery was between 85 and 100%. A, DNA from silets incubated without streptoxotoch for 100 min (a) and with streptoxotocm (2 mM) for 5 min (b), 10 min (c) and 20 min (d).  $\blacksquare$ , DNA from silets incubated without alloxan for 10 min (a) and with alloxan (1 mM) for 5 min (f), 10 min (g) and 20 min (h). Sedimentation was from left to right. Arrow indicates the position of a bacteriophage  $\lambda$  DNA (3.2×10<sup>7</sup> molecular weight, New England BioLabs).

Fig. 2 Effect of streptozotocin or alloxan on poly(ADP-nbose) synthetise activity and NAD content of pancreatic islet cells. Batches of 300 islets were incubated for 0-60 mm at 37 °C in conditions described in Fig. 1 legend. After incubation, nuclear fractions were prepared from islets and assayed for poly(ADP-ribose) synthetise activity as described previously? The DNA content of each islet nuclear fraction was determined by a fluorimetric method. The properties activity as described previously? The DNA content of each islet nuclear fraction was determined by a fluorimetric method. The properties activity at 0 mm (10.9 pmol poly(ADP-ribose) synthesized per 10 mm per μg islet nuclear DNA). a, Poly(ADP-ribose) synthetises activity in islets incubated with 2 mM streptozotocin (Δ); b, poly(ADP-ribose) synthetises activity in islets incubated with 1 mM alloxan (O). For determination of silet NAD content, batches of 100 islets were incubated for 0-60 min after which they were disrupted by sonication in 1 ml of cold 0.5 M perchloric acid (PCA). The scad-soluble extract of islets was brought to pH 5 0 with KOH, and NAD content in the extract, determined as described elsewhere. The values were corrected for overall recovery by the addition of 14C-NAD (see Table 1 legend), and related to NAD content at 0 min (2.62 pmol NAD per sitel). c, NAD content in islets incubated with 1 mM alloxan (②).

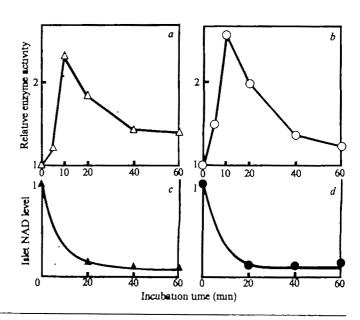


Table 2 Effect of nicotinamide or picolinamide on depression of proinsulin synthesis induced by alloxan or streptozotocin

Addition	<sup>3</sup> H-proinsulin synthesized (c.p.m. per h per islet)
None	3,410 (100)
Alloxan (1 mM)	307 (9)
Alloxan (1 mM) and nicotinamide (2 mM)	1,194 (35)
Alloxan (1 mM) and picolinamide (2 mM)	921 (27)
Alloxan (0.5 mM)	1,009 (30)
Alloxan (0.5 mM) and nicotinamide (2 mM)	3,372 (99)
Alloxan (0.5 mM) and picolinamide (2 mM)	2,496 (73)
Streptozotocin (2 mM)	1,187 (35)
Streptozotocin (2 mM) and nicotinamide (2 mM)	2,868 (84)
Streptozotocin (2 mM) and picolinamide (2 mM)	2,568 (75)
Streptozotocin (1 mM)	2,114 (62)
Streptozotocin (1 mM) and nicotinamide (2 mM)	3,096 (91)
Streptozotocin (1 mM) and picolinamide (2 mM)	3,045 (89)
Nicotinamide (2 mM)	3,584 (105)
Picolinamide (2 mM)	3,342 (98)

Batches of 30 islets were preincubated at 37 °C for 5 min in 100 µl of Krebs-Ringer's bicarbonate medium containing 2.8 mM glucose in the presence or absence of the chemicals as indicated, then incubated for 60 min with the addition 100 μl of medium containing 10 μCi <sup>3</sup>H-leucine (specific activity 122 Ci mmol<sup>-1</sup>; NEN) and 37.2 mM glucose. The final concentration of glucose was 20 mM, at which proinsulin comprises more than one-half the total proteins synthesized de novo in islets and gives a major peak on SDS-polyacrylamide gel electrophoresis<sup>22</sup>. Incubated islets were washed three times with cold Hank's solution, sonicated in 200 µl of 60 mM Tris-phosphoric acid buffer, pH 6.8, containing 1% SDS, 5% 2-mercaptoethanol and 10% glycerol, and heated at 100°C for 2 min. An 80-µl aliquot of the heated sample was submitted to SDS-polyacrylamide gel electrophoresis. The amount of proinsulin synthesized was calculated by summing the <sup>3</sup>H radioactivity incorporated in the proinsulin fractions<sup>22</sup>. Numbers in parentheses are values for proinsulin synthesis as a percentage of that in islets incubated without any additions.

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# Intramolecular flexibility in phenylalanine transfer RNA

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Yeast tRNAPhe is an L-shaped molecule, where each arm is a short segment of double-helical RNA. Examination of the crystal structure has suggested that the molecule may be hinged, perhaps in the region where the anticodon stem meets the D stem1, or where the two arms of the molecule meet2. Because tRNA interacts with a variety of macromolecules during protein synthesis, and because it performs various functions in addition to its role in protein synthesis<sup>3</sup>, the idea of a hinge is attractive: such conformational flexibility would facilitate functional flexibility. Here we present the results of a theoretical study of the bending of  $tRNA^{Phe}$  about the proposed hinge at the junction of the two arms2, applying the conformational energy calculation method used previously to examine flexibility in lysozyme4 and in DNA5. The model is surprisingly flexible, swivelling with two degrees of freedom through angles as large as 30° at an energetic cost of only a few kcal per mol. These energies are comparable with that of a few hydrogen bonds, suggesting that solvent conditions and interactions with other molecules are important in modulating structure and flexibility.

The molecule is modelled on a computer by specifying the coordinates of all the atoms as determined crystallographically, the molecular topology (which atoms are bonded to each other) and a set of empirical energy functions<sup>6,7</sup> for all the bond lengths, bond angles, dihedral angles, nonbonded contacts (both van der Waals and electrostatic) and hydrogen bonds. Then, treating the two arms as essentially rigid and specifying the location of the hinge or swivel between them, one arm is rotated about that point in increments of 3°; partial structural relaxation is allowed by energy refinement after each bending increment. The resulting sets of coordinates are subjected to several hundred cycles of steepest-descent energy refinement, and the difference between

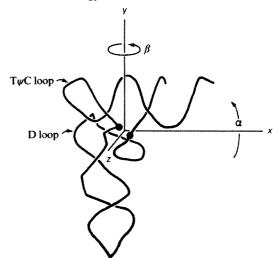


Fig. 1 Backbone of tRNA<sup>Phe</sup>, showing D and TψC loops, coordinate system centred between phosphates P8 and P49 ( ) and the two degrees of freedom. The coordinate system is not that of the crystal structure but has been chosen so that the xy plane is the least-squares best fit plane in the sense that the sum of the squares of the atomic distances from the plane is a minimum. True bending is represented by  $\alpha$ , measuring rotations about the z axis, while  $\beta$ designates swinging, with rotations about the y axis. Positive values of  $\alpha$  swing the arms apart and push the D and T $\psi$ C loops together; positive values of  $\beta$  keep the arms at right angles and pull the D and  $T\psi C$  loops apart.

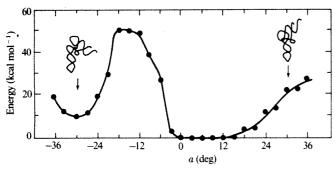


Fig. 2 Dependence of energy on bend angle  $\alpha$ . The crystallographic structure defines  $\alpha=0^\circ$  and the backbone conformations are indicated for  $\alpha = \pm 30^{\circ}$ . The swing angle is unchanged  $(\beta = 0^{\circ})$ during the bending. In Figs 2 and 3, for clarity, negative energies are shown as E = 0; no values below -2 kcal mol<sup>-1</sup> were observed. and because this is about the limit of resolution and the scatter was random (no clear minima were observed), we do not consider the negative values to be significant.

the energies of the bent and unbent models is assumed to be the energy cost of bending.

For this study, we used the 2.5 Å crystallographic structure of Jack et al. 7,8 obtained from the Brookhaven Protein Data Bank9. The empirical energy parameters are essentially those of Levitt<sup>5,16</sup>, as subsequently modified for tRNA<sup>7</sup>, but partial electrostatic charges<sup>11</sup> and approximate corrections for solvent dielectric effects<sup>12</sup> have been included. Neither Mg<sup>2+</sup> nor any other cation has been included, as the study was intended to assess the intrinsic molecular flexibility; we hope to examine the effects of polyvalent cations in future work. There are 79 hydrogen bonds in the model, including the 56 interbase bonds of the cloverleaf secondary structure, 12 bonds between O2' of one ribose and an oxygen atom (O4' or O5') of the successive ribose, and 11 tertiary bonds. This represents all the commonly accepted hydrogen bonds<sup>13</sup> except for 10: 7 bonds between the D and T $\psi$ C loops (2 between G18 and  $\psi$ 55; 3 between G19 and C56; 1 between G57 and R18; 1 between G57 and R19) and 3 within the T $\psi$ C loop (2 between T54 and A58; 1 between G57 and R55). The interloop bonds are proposed to serve as a catch mechanism which opposes bending, while the bonds within the  $T\psi C$  loop were found to become highly distorted as the loop folds to accommodate the bending motions. As these 10 tertiary hydrogen bonds are believed to be among the most sensitive to changes in temperature, pH, ionic strength and concentration of polyvalent cations<sup>14</sup>, they are likely candidates for modulating flexibility. Breaking these bonds requires 22 kcal mol<sup>-1</sup>

After a preliminary examination of various bending motions about several prospective points, we concentrated on the softest bending modes (Fig. 1). The molecule swivels about the region near P8 and P49, with bases 1-7 and 49-76 comprising the upper arm and bases 8-48 comprising the lower. As movement arises from the distortion of several bond angles and dihedrals and the energy minimization algorithm relaxes those distortions by moving several neighbouring atoms, the swivel is not truly a discrete point. Two macroscopic degrees of freedom have been examined, corresponding to the angles  $\alpha$  (true bending, with the upper arm rotating about the z axis relative to the fixed lower

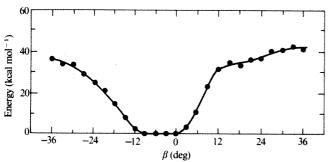


Fig. 3 Dependence of energy on swing angle  $\beta$ , with  $\alpha = 0^{\circ}$ .

arm) and  $\beta$  (swinging, with the upper arm rotating about the y axis while keeping at a right angle to the lower arm). In this study, these two degrees of freedom have been examined separately; a detailed analysis of motions which combine bending and swinging will be reported elsewhere.

Figures 2 and 3 show the results, giving the calculated potential energies for bending and swinging respectively. The most surprising result is that the molecule swivels over an appreciable range of angles using relatively little energy: for <3 kcal mol<sup>-1</sup> it covers a range of about 15° in each direction, while over 30° is covered for <20 kcal mol<sup>-1</sup>.

Because these energies represent less than that contained in 10 hydrogen bonds, a number which could easily be made or broken in interactions either with the solvent or with other macromolecules, such interactions clearly could modulate both the overall shape and flexibility of the molecule. While these results do not prove that tRNA Phe is flexible (especially as the solvent is not explicitly treated in the model), they do suggest that there are no large steric or electrostatic forces within the molecule which oppose swivelling. Consequently, we consider it quite likely that tRNAs sometimes have conformations quite different from the familiar L shape and that flexibility can be induced by moderate changes in solvent conditions.

Also interesting is the anisotropy of the forces which oppose the swivelling. The strongest forces (the steepest slopes in Figs 2, 3) are those resisting closing ( $\alpha < 0$ ) and swinging in one direction  $(\beta > 0)$ ; they arise from the fact that these two motions pull apart the stack of bases G19-G57-G18-m<sup>1</sup>A58 that stabilizes the interaction between the D and T $\psi$ C loops, whereas motions in the other directions ( $\alpha > 0$  and  $\beta < 0$ ) maintain that stacking. The 35-55 kcal mol<sup>-1</sup> required to break the stack of bases is an upper limit, as our steepest-descent minimization algorithm probably does not fold the loops into their lowest-energy conformations as they are separated, but it is approximately what would be expected for a stack of four purines, with base stacking interactions of 5-15 kcal per mol per stacked pair 15.1

During the closing motion, the energy drops below  $\alpha < -18^{\circ}$ , as the stacks G19-G18 and G57-m1A58 are formed and as favourable nonbonded interactions are obtained in the inside of the elbow, where many atoms are brought together by the motion. As a more careful folding of the molecule during bending might substantially reduce the height of the energy barrier in Fig. 2, our results give a conservative estimate of the intrinsic flexibility of tRNA<sup>Phe</sup>, and the molecule may bend even more easily than indicated here.

The flexibility revealed by this study is consistent with the thermal motion observed by crystallographers 7,17 , who reported that atoms near the ends of the arms move more than those near the elbow. The specific biological functions of this flexibility remain to be established, but these motions may facilitate interactions between tRNA and other macromolecules, and tRNA may assume different conformations at different steps in protein synthesis.

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# MATTERS ARISINO

#### Importance of cardiac cell membranes in vanadateinduced NADH oxidation

WE have reported previously that purified cardiac cell membranes with high (Na++K+)ATPase activity have significant NADH-vanadate reductase activity. In our experiments, we used calf cardiac cell membranes prepared as described elsewhere<sup>2</sup>, but similar results were obtained with cell membranes from cat and human ventricles and from human erythrocytes. Increasing concentrations of Na<sub>3</sub>VO<sub>4</sub> (0.01-1 mM) in the presence of 10 mM imidazole/HCl buffer pH 7.25, 1.25 mM NADH and 0.1-0.2 mg cell membrane protein caused a concentration-dependent oxidation of NADH (followed photometrically by continuous recording at  $A_{366}$ ), which was virtually absent when heat-denatured cell membranes were used. A more detailed analysis of these experiments has been published elsewhere3

Recently, Vyskočil and co-workers<sup>4</sup> reported that vanadate-induced oxidation of NADH does not require a specific enzyme. Moreover, they did not observe a vanadate-dependent oxidation of NADH in the presence of membrane fractions from rat heart and other tissues (skeletal muscle, brain and liver). They found that NADH oxidation in the presence of vanadate proceeded without cardiac cell membranes in the presence of several buffers, except imidazole/HCl, in which the reaction was completely inhibited. Although so far we agree with these results of Vyskočil et al.4, we tested the vanadate-NADH reaction using rat heart membranes and again found vanadate-dependent oxidation of NADH in the presence of membrane fractions. Thus we maintain that cardiac cell membranes (also from rat) contain a heatsensitive NADH-oxidoreductase that is stimulated by vanadate in the presence 10 mM imidazole/HCl. Several authors<sup>5-7</sup> have found a NADH-oxidizing enzyme in plasma membranes of rat liver, rat skeletal muscle and other organs of various species.

A possible reason for the failure of Vyskočil et al. to detect the vanadatedependent oxidation of NADH in the presence of membrane fractions from rat heart might be the procedure used to isolate the membranes or the preparation of the vanadate solution. We used a solution of Na<sub>3</sub>VO<sub>4</sub> that was freshly prepared on each day of the experiment in twice distilled water and adjusted to pH 7.25 with HCl.

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VYSKOČIL ET AL. REPLY—The following remarks are pertinent to the discrepancies between our experimental data<sup>1</sup> and those of Erdmann et al.<sup>2</sup>.

First, the preparation of the vanadate solutions may well have caused some of the differences. In previous papers<sup>2</sup>, Erdmann et al. did not describe the preparation of the vanadate stock solutions; other papers3-6 have shown that the degree of polymerization of vanadate species depends on the pH and concentration of vanadate in the solution, and on temperature and ionic strength. Particularly at vanadate concentrations of 0.1-10 mM) and within the pH range 7.0-8.0, it is uncertain which species predominates, as dimeric, trimeric and decameric clusters may be formed. The possibility cannot be excluded that Erdmann et al. worked with vanadate solutions in which decameric anions prevailed, even when freshly prepared. However, we observed no effect in imidazole solution when a stock solution of vanadate (V) was made with NaOH7. On the other hand, in the decavanadate solution prepared according to Choate and Mansour4, we also observed vanadate-dependent oxidation of NADH in the presence of rat skeletal muscle membrane fractions in an imidazole buffer. It is possible (see ref. 6) that membrane fractions promote the NADH-vanadate (V) reactions by preferential binding of reduced vanadyl (IV), which is consistent with Rubinson's statement<sup>6</sup> that if free ligand(s)—probably some thermolabile components of membranes—are present in a pHbuffered solution, then vanadate (V) ions will tend to be reduced to vanadyl (IV) forms (which bind to the ligand more strongly than the V form) provided there is some electron-donating reagent such as NADH.

Moreover, in an imidazole-buffered medium, most vanadate (v) can be chelated preferentially with imidazole rather than with NADH. In this case, the NADH-vanadate redox reaction (which

proceeds in other buffers within the NADH-vanadate complex<sup>1,7</sup>) is stopped and needs to be triggered by other ligands (for example, by flavines, F.V., H.D. and J.T., unpublished results).

Another possible source of discrepancy between our results and those of Erdmann et al. may have resulted from different treatment of membrane fractions with sodium iodide8. Other facts must be taken into account, such as the stability of the preparation<sup>2</sup> and the possibility of contamination of the cytoplasmic dehydrogenases, which can be attached to the membrane8.

For these reasons we believe that within the cell, the oxidation of NADH by vanadate may proceed by forming a complex without any additional support from a specific enzyme, but we do not doubt the validity of the data obtained by Erdmann et al. in their specific experimental conditions.

Note added in proof: The rapid nonenzymatic oxidation of NADH (10<sup>-3</sup> M) by vanadate (10<sup>-3</sup> M) in phosphate buffer (pH 7.0) is accompanied by the appearance of equivalent amounts of vanadyl(IV) as revealed by the ESR method (Pilař, Vyskočil, Dlouhá and Teisinger, in preparation).

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#### Lower Palaeozoic oceans and Wilson cycles

ANDERTON1 has recently suggested that Iapetus (the ocean separating north-west and south-east Britain in the Lower Palaeozoic) may have opened as late as the beginning of the Cambrian. There seems to be a general concensus now that these two parts of Britain were brought together in late Silurian-early Devonian times and that an ocean between them-Iapetus-was thereby destroyed. It has been almost tacitly assumed since the early models of Wilson<sup>2</sup> and Dewey<sup>3</sup> that this ocean must have opened some time before this. Most models (including my own4) have assumed that an ocean existed between north-west and south-east Britain for a considerable period. But, from palaeomagnetic reconstructions of the late Precambrian and early Palaeozoic<sup>5,6</sup>, Iapetus was probably not a two-sided ocean comparable with the Atlantic and there therefore seems little justification for trying to narrow down the timing of opening of a non-existent ocean.

There are few reliable constraints on the relative latitudinal positions of the two sides of the Caledonides in late Pretimes. One interesting cambrian palaeomagnetic result suggests that the Baltic Shield may have lain to the southeast of Scotland in late Grenville times7. This position would make the similarities between the Scottish Dalradian and the northern Norway late Precambrian more easily understandable and the 'opening' suggested by Anderton<sup>1</sup> may be allied to the separation of the Baltic and Laurentian shields. This would be regarded as the 'opening of Iapetus' by Scandinavian geologists but is a different concept from that generally envisaged in Britain and it must be remembered that the original designation of Iapetus<sup>8</sup> was for the Svalbard-Scandinavia-Greenland segment of the Caledonides. Faunal differences between south-east Britain and Scotland thus become irrelevant to the opening of that ocean.

The position of south-east Britain in late Precambrian and early Palaeozoic times is essentially unknown, but the combination of a distinctly different Precambrian structural history between the two sides and the widely different Lower Cambrian faunas suggests that north-west and south-east Britain were not in juxtaposition at any time before the final closure of Iapetus. Palaeogeographical models<sup>5,6</sup> have all put south-east Britain at the edge of completely different continental masses from Scotland-and not on the 'other' side of a linear ocean. It is only by rotations and large scale lateral translations (along major transforms of the ocean floor<sup>6</sup>) that the two sides eventually came together at the end of the Silurian. The Iapetus Ocean thus only existed as a twosided closing ocean for probably the last part of Ordovician and Silurian times, at least in the Britain-Appalachia segment of the Caledonides.

reconstructions suggest Many completely different patterns of movement for the various sections of the southeast margin of the Caledonian orogeny. Morocco, Avalon (Newfoundland)/New England, Iberia/Brittany, England/ Wales, central Europe and Scandinavia may all have moved independently from widely separated positions on the globe to their final resting place against the more or less uniform north-west margin of Greenland-Scotland-northwest Newfoundland-Appalachia. This is fairly unlikely in that one might not expect several microcontinental fragments to collide at even approximately the same time to form the Caledonide orogeny (even allowing for the now well recognized differences in time of collision between Scandinavia and north-west England<sup>9</sup>). There is no evidence that these microcontinental fragments show any evidence of collision with each other before the final closure of The fact that Morocco, Iapetus. Avalon/New England, south-east Britain and Brittany/Iberia also show evidence of a similar late Precambrian history at the margin of a continent suggests that these fragments may have lain along one continental margin<sup>10</sup> which gave rise to the Cadomian cordilleran-type continental margin orogeny.

The sediments of the central European area of this age are marine, probably not shelf sediments, and include a considerable proportion of submarine volcanics, but again the geotectonic nature of the volcanicity and its significance are uncertain. However, it still seems likely that a late Precambrian-early Palaeozoic ocean margin lay along the line of the central European Palaeozoic massifs.

Thus the closure of Iapetus at the end of the Lower Palaeozoic seems a good example of the end of a Wilson cycle. But the late Precambrian and Cambrian history of the two sides of the closing ocean suggests that the opening phase of the Wilson cycle—the splitting apart of the continental mass-probably only affected Scandinavia and Laurentia. In this respect the suggestion<sup>11</sup> that the 560-Myr alkaline volcanic<sup>12</sup> and rift system of the North Atlantic may be an indication of the initial splitting may be correct. These volcanics and graben structures occur in Canada, South Greenland and near Oslo on the Baltic Shield, while the Carn Chuinneag granite of the Scottish Moine area, which is also an alkaline pluton 13 of this age<sup>14</sup>, may well be a Scottish equivalent of this suite. However, the suggestion that the Charnwood Forest volcanics also belong to this event, and even that it lies in a rift structure9, are not based on any real evidence. In fact all the volcanics on the south-east side which

have been suggested as remnants of the initial splitting probably belong to a volcanic arc system resultant from subduction under this continental margin9.

The whole concept of a Wilson cycleof continental splitting, ocean widening and continental closure—thus takes on a new light. Study of the closing oceans of many areas through geological time, now made possible by the palaeomagnetic and palaeogeographical compilations of Smith et al.1 and Zeigler et al.5, reveals that collisions are not between many continental fragments that had previously been even approximately adjacent, although neither goes far enough back into the Precambrian to describe the early portions of the two sides of Iapetus.

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ANDERTON REPLIES-I agree with Wright about the need to reconsider the Wilson cycle and most of his points about the plate tectonic evolution of the British Isles. As I have discussed the opening of Iapetus in some detail elsewhere<sup>1</sup>, I shall just summarize my present views.

I agree that during the Precambrian, north-west and south-east Britain evolved in isolation from each other and that the late Silurian/early Devonian continental collision brought them together for the first time. However, the similarities between Laurentia and Baltica suggest that these areas were part of the same plate during the late Precambrian (although not necessarily in their post-Caledonian relative positions), that this plate split at the end of the Precambrian or in early Cambrian times to form the Iapetus Ocean and that the ocean subsequently dosed. Thus, although it does not make sense to talk about the opening of Inpetus in the restricted

context of the British Isles, it may well make sense in a broader, North Atlantic context. However, as Wright implies, the suggested opening and closing of Iapetus between Laurentia and Baltica may well be the exception in plate tectonic behaviour, and the collision of previously unrelated plates, as exemplified by the British Isles, be the rule.

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1. Anderton, R. J. geol. Soc. Lond. (in the press).

of their procedures on just one or a few of these variants?

Our own unpublished observations indicate that all of a variety of tested W compound mice lack cells staining with toluidine blue in both skin and intestine. The mice tested include  $W^{sh-2}W^{sh-2}$  (ref... 8), which apart from being black-eyed whites are otherwise substantially normal. Thus the W locus seems to control 'toluidine-blue-affin' cells as well as pigment migration.

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#### Spleen colony-forming cell as common precursor of tissue mast cells and granulocytes

THE demonstration by Kitamura and his colleagues1 of the origin of mast cells from CFU-S is persuasive but to us incomplete. Recipient  $W/W^v$  mice with great paucity of mast cells and CFU-S, nevertheless have stem cells which support haematopoiesis and rescue lethally irradiated mice<sup>2,3</sup>. Donated hemi-syngeneic stem cells from single splenic colonies of normal and beige mice must be few, on average of the order of 10 (ref. 4); only a minority of colonies from the tail of the skew distribution have 100 or more which may be necessary to father without mitotic exhaustion<sup>5</sup> a viable population of donated haematopoietic cells so as to compete with the host's established haematopoiesis. Kitamura et al. claim 23/106 such occasions (Table 1 of ref. 1); but their Table 2 identifies only 2/106 where erythropoiesis was demonstrable in addition to a raised population of 'mast' cells

Indeed we question whether mast cells have been demonstrated at all. They used as their criterion cells stained with toluidine blue. The 23 reported cases with whole body distribution are highly biassed by cells in spleen. It could be that these are basophil granulocytes of myeloid tissue, possibly but not necessarily equated with tissue mast cells. Note that none of the 106 animals showed mast cells in skin, which is repopulated by donor mast cells when compound W mice are repopulated with a generous supply of normal stem cells (ref. 6 and our unpublished results).

Others7 have drawn distinctions not mentioned here between skin and intestinal mast cell populations. Is it not likely that cells staining with toluidine blue are quite heterogeneous and that Kitamura et al. are observing the effects Kitamura, Y., Yokoyama, M., Matsuda, H., Ohno, T. & Mori, K. J. Nature 291, 159-160 (1981).

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KITAMURA AND *YOKOYAMA* REPLY-We have reported that the spleen colony-forming cell is a common precursor of tissue mast cells and neutrophil granulocytes1. We used mice of two mutant genotypes in the experiment. Giant granules of  $bg^{J}/bg^{J}$  mice were used for identification of the origin of both tissue mast cells and neutrophils, and  $W/W^{v}$  mice were useful recipients because they lack tissue mast cells.

Loutit et al. have pointed out a discrepancy between the proportion of mice in which mast cells appeared and that of mice in which donor-type erythropoiesis was demonstrable. As they mentioned,  $W/W^{v}$  mice have haematopoietic stem cells but their function is apparently abnormal. Numbers of neutrophils, erythrocytes and tissue mast cells in adult  $W/W^{v}$  mice are respectively ~100, 50 and 1% of the value in congeneic +/+mice<sup>2,3</sup>. The mechanisms which determine such differentiation patterns of abnormal stem cells in  $W/W^{\circ}$  mice are unclear. However, the haematological situation in  $W/W^v$  hosts may explain the discrepancy discussed by Loutit et al. The transplanted normal stem cells might be selectively committed to tissue mast cells rather than to neutrophils or erythrocytes.

We think that the 'toluidine-blue-affin' cells counted in our experiments are true mast cells for the following reasons. (1) The cells were not only stained with toluidine blue but also contained

histamine and heparin. The histamine concentration in the forestomach4 and the skin<sup>5</sup> of  $W/W^{\circ}$  mice is ~1% the value observed in +/+ mice. Heparin is not demonstrable in the skin of  $W/W^{v}$  mice<sup>6</sup>. (2) The toluidine-blue-affin cells do not seem to be basophil leukocytes on the basis of their morphology and mode of differentiation. Typical mast cells can be differentiated from typical basophils by morphological criteria7. Most toluidineblue-affin cells which appeared in the spleen of  $W/W^{v}$  mice after transplantation of spleen colony-forming cells were typical tissue mast cells. Moreover, no typical basophils were detected in smears of the bone marrow, spleen or peripheral blood of mice of any genotypes. Basophils, as well as neutrophils and eosinophils, seem to leave haematopoietic tissues after maturation. In contrast, precursors of our toluidine-blue-affin cells proliferated in connective tissues before final differentiation is characteristic of tissue mast cells8,9

The fact that there was no increase in mast cell number detected in the skin of  $W/W^v$  mice after injection of cells from a single spleen colony may be attributable to the differential demands of mast cell differentiation between tissues. Donortype mast cells appeared in the caecum and stomach but not in the skin when bone marrow cells of  $bg^{J}/bg^{J}$  mice were injected into irradiated +/+ mice<sup>10</sup>. However, the origin of the intestinal mast cells was not different from that of skin because donor-type mast cells also appeared in the skin after local applications of methylcholanthrene to such radiation chimaeras9. Moreover, mast cells appeared in the caecum and stomach of  $5/9 W/W^{v}$  mice but in the skin of only 1/9 W/W" mice after transplantation of a small number (104) of bone marrow cells from the +/+ donors<sup>11</sup>, this mouse was also cured of anaemia<sup>11</sup>.

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A comprehensive discussion and review of the prevention and management of anaerobic bacterial diseases including clost-ridial infections and non-clostridial anaerobic infections including post-surgical sepsis. A full bibliography is included. Series: Antimicrobial Chemotherapy Research Studies, Vol. 1 0471 28037 2 106pp July 1981 \$26.60/£9.50 Research Studies Press: A Division of John Wiley & Sons Ltd.

## Electron Microscopy in Biology, Vol. 1

edited by J.D. Griffith, University of North Carolina, Chapel Hill Discusses current techniques and biological applications of electron microscopy, serving as a unifying reference for various subdisciplines. Topics in this first volume include image processing, freeze-etching of chloroplast membranes, chromatin ultrastructure, visualization of RNA, and visualization and mapping of genes by the R-loop technique. It also includes critical interpretations of electron micrographs.

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## Biology of Carbohydrates, Vol. 1

by V. Ginsburg, National Institute of Health, Maryland

Presents authoritative discussions of selected topics by leaders in the field. Subjects are selected for their timeliness and potential for eliciting intelligent generalizations and worthwhile speculations.

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## The Sarcoplasmic Reticulum: Transport and Energy Transduction

by L. de Meis, Federal University of Rio de Janeiro

Discusses the mechanisms of Ca²+ transport and energy transduction in isolated sarcoplasmic reticulum vesicles. It raises the possibility that the sarcoplasmic reticulum might interconvert binding energy into chemical energy. The book shows that, under suitable conditions, the entire process of Ca²+ transport can be reversed and the very same ATPase involved in Ca²+ transport is able to catalyze the synthesis of ATP in a process coupled with the release of Ca²+.

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## **BOOK REVIEWS**

## I and brain

## **Donald Michie**

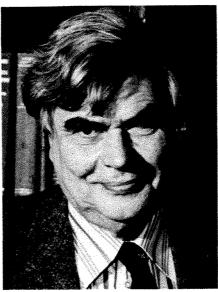
CONCERNING ways in which language limits perception, Paul Henle has observed:

It would seem then to be consistent with what we know of mental set on other grounds to assume that the world appears different to a person using one vocabulary than it would to a person using another. . . . The Navaho, for example, possess colour terms corresponding roughly to our 'white', 'red', and 'yellow' but none which are equivalent to our 'black', 'grey', 'brown', 'blue', and 'green'. They have two terms corresponding to 'black', one denoting the black of darkness, the other the black of objects such as coal. . . . As far as vocabulary is concerned they divide the spectrum into segments different from ours.

The passage is quoted in a new book by our most prominent student of the physiology and psychology of perception, who moreover brings to it an unusual insight into the science of mechanism: Richard Gregory is also a competent and highly inventive engineer.

A decade or more ago his Eye and Brain confirmed for perception what Martin Gardner's Logic Machines and Diagrams had earlier shown for deductive thought first, that analysis of brain-mediated operations prospers better on a machineoriented than a metaphysical base; and second that brilliant popularization is possible without loss of rigour. The intellectual vocabularies which Gregory brought to bear in Eye and Brain were those of neurobiology and engineering physics. Both are native to him as a scientist, and they were precisely the two which the task demanded. The new enterprise, Mind in Science, far out-demands the old, being no less than a survey from earliest times of the effects of technology upon explanatory theories in science.

The requirement for a multi-lingual intellect imposed by such a task seems aweinspiring. The book's 600 pages sweep through the origins of myths and of tools, the invention and nature of writing, of mathematics and of machines; through natural philosophy from pre-Socratic to post-Einsteinian; through early notions of biology and medicine to the origin of species and to the descent and cultural ascent of man; to the measurement and definition of intelligence, the philosophy of mind, the physiology of brain, the psychology and metaphysics of perception, and finally to language, consciousness and the nature of knowledge and truth. Interwoven through the narrative are strands of sociology, mechanics, thermodynamics, cybernetics (this last is genially labelled by Gregory's broad brush as "Artificial Mind in Science: A History of Explanations in Psychology and Physics. By Richard L. Gregory. Pp.641. ISBN UK 0-297-77825-0; ISBN US 0-521-24307-6. (Weidenfeld & Nicolson/Cambridge University Press: 1981.) £18.50, \$29.95.



Richard Gregory — a good companion

Intelligence") and of symbolic logic.

Equally breathtaking is how close Gregory comes to success. It is as though some Leonardo of the Navaho had essayed a treatise on pigments of many lands, and had almost accomplished the heroic act while still, to return to Henle, having but a single term for "grey" and "brown", and another for "blue" and "green". The mental vocabulary missing from Gregory's near-masterwork is that of computation, interpreted broadly to include the principles of modern predicate logic and the means of implementation adopted by the designers of the new generation of intelligent computing systems. In the result, whenever we follow the author to the active modelling of thought by machine, our thirst intensifies; but it is at these very points that the stream dries.

The abstract forerunner of the modern computer is A.M. Turing's startling and consequential theoretical construct, the Universal Turing Machine. One might expect this historic intellectual mechanism to figure prominently in a book with such a theme as Gregory's. It comes as a surprise to find it boiled down to "... the important notion advanced by the pioneer computer scientist at Manchester, Alan

Turing: that anything that is conceivable can be represented by a simple machine, capable of a very long string of alternative states". The "important notion" was in fact advanced in the mid-1930s when Turing was at Cambridge. When he moved at Max Newman's invitation to Manchester in September 1948 it was not to "found the Manchester computing laboratory" as stated later in this book (p.382). By that time Tom Kilburn and F.C. Williams had already achieved operation of the Manchester prototype on which they had embarked in December 1946. But more serious is the author's failure to grasp, and hence to communicate, what the whole Babbage-Turing-Kilburn-Williams-Wilkes-Eckert-Mauchly-von Neumann story was actually about.

Richard Gregory has one of the most richly furnished and dazzling minds that I have ever encountered. But for every person, there are one or two subjects which run so widdershins to the natural grain as to become subtly repellent, almost "antisubjects". For myself, however long and intently I strive, I cannot learn a foreign language. Gregory cannot learn a computer language. At Edinburgh he was briefly enticed into the attempt. But the terminology of tests and loops seemed to grate so painfully that the experiment was dropped. Here is a clue to what can make a scholar attached to historicity and precision go awry. When the anti-subject returns to mind, repellency is re-awakened and the instruments of memory are jarred.

I was captured by the dream of AI while it was yet a seed without flower or fruit: to leave Cambridge to help my colleagues Donald Michie and Christopher Longuet-Higgins to set up the Department of Machine Intelligence and Perception at Edinburgh University. Here we collaborated with the corresponding Departments at MIT and Stanford, and we built the Robot Freddy, which was one of the first to recognise objects from any point of view and to make models.

A meccano mounting of a TV camera on two small motor-driven wheels had indeed been used by Barrow and Popplestone (see their subsequent 1971 paper in *Machine Intelligence* 6) for their own preliminary skirmish with the vision problem before Gregory's departure to Bristol in September 1970. But the robot to which he must surely be referring was then still at the planning stage, to be completed, and its computer-based accomplishments demon-

strated, 2½ years later. The team which saw the Freddy project through consisted of A.P. Ambler, H.G. Barrow, C.M. Brown, R.M. Burstall and R.J. Popplestone (see their paper in *Artificial Intelligence* Summer 1975); and on the hardware side S. Salter (mechanical engineering) and Gregan Crawford (electronics).

It should also be recalled that the founding giants of AI, men such as Samuel, Simon, Newell, McCarthy, Minsky, Robinson and Rosen had been labouring to set the cornerstones some ten years before Gregory was captured by his dream of flower and fruit. But the bold literary approximation sometimes economically conveys a deeper truth. Before he left Edinburgh Gregory taught us that if a robot is to see, then its programmers must enable it to reconstruct its percepts, as does the human being, from a stored inventory of how the components of familiar objects and scenes normally look. This Helmholtzian doctrine is a cliché of computer vision today. In those days it was not, and the launching orientation was derived, to speak for myself, in powerful measure from Gregory.

From time to time in the book this gift flashes forth to astonish, illuminate and (occasionally) to infuriate. It is human to be irked by an inspired guide who against overwhelming odds brings one to the right door, only to signal by tell-tale fumblings that he lacks the door key. But is it rational to be irked? Who else could have piloted one thus far? Moreover one is perfectly free to thank him and look for a key-owner or a locksmith. Gregory has seen from afar that the instruments of thought-automation are the "tools", "materials" and "processes" of a new industry, the technology of human knowledge. On approaching closer, his expertise gives out, just where that of the new race of knowledge engineers begins. The important thing, however, is that his insight is correct.

The book's pivotal chapter, "The Nature and Nurture of Intelligence", will stir profitable thought in laboratories where man-machine synthesis of prototype knowledge systems is making precise what Gregory's mind's eye has spied. As with the book as a whole, the chapter tells everything about the philosophy of AI, nothing of the method.

It also tells on every page a very great deal about its author. From this comes many flaws, but much charm. One of the joys of the scientific life is to drop for a while the customary pendantries in exchange for good companionship. Mind in Science offers sustenance of just this kind. Possibly some scientific or philosophical people may abstain from its pages. They must be warned that they will have to continue their journey more poorly supplied than need have been the case.

Donald Michie is Professor of Machine Intelligence in the University of Edinburgh.

## Cell transformation: a missed opportunity

Daniel Rifkin

Neoplastic and Normal Cells in Culture. Developmental and Cell Biology, 8. By J.M. Vasiliev and I.M. Gelfand. Pp.372. ISBN 0-521-23149-3. (Cambridge University Press: 1981.) £40, \$79.50.

THE enormous increase of interest over the past ten years in the molecular biology of tumour viruses, as well as in the physiology of cultured cells, has created an extensive literature on the properties of normal and transformed cells, and on the relationships of these properties to the cellular phenotype. There is no book currently available which brings together the large body of information accumulated in this area. Thus, it was with pleasure that I began Vasiliev and Gelfand's book Neoplastic and Normal Cells in Culture. The aim of the authors is "to describe and discuss comparative characteristics of the interactions of normal and neoplastic cells with their environment in cell cultures". The book, then, would appear to be the perfect vehicle for a synthesis of data on those properties of neoplastic cells responsible for their altered growth, their invasive behaviour and their metastatic potential, specifically as measured by in vitro criteria.

The book is divided into four parts. Part 1, an introduction, is subdivided into two sections: the first covers the general properties of neoplastic cells, especially growth, and the second deals with neoplastic transformation in culture by a variety of agents. The second part of the book, "Morphogenesis in 'Normal and Transformed Cultures'," is concerned with cellular structures and their function in relation to morphogenesis. This includes the movement of normal fibroblasts and epithelial cells in relation to their morphology, the locomotion and morphology of tumour cells, and specific biochemical or cytological processes whose alteration may be responsible for the difference between normal and transformed cells. Part 3 deals with growth regulation in normal and transformed cells, concentrating on the anchorage, density and soluble factor controls of cell division, and the interactions of these phenomena. The book concludes with a general discussion based on a comparison of in vitro and in vivo malignant cells as well as the interrelationships of transformation properties.

Within the scope of the authors' aims, the book is filled with interesting references and intriguing observations. The authors present a number of thoughtful conclusions and certainly point out certain ambiguities in the data from different laboratories.

However, the book has two serious deficiencies. The first of these is the cursory discussion of the possible significance of

cell-matrix interactions in determining the cellular phenotype. The possible influences of fibronectin, collagen and proteoglycans on cell behaviour are given little more than two paragraphs. This is a rather exciting area of current experimentation but it is presented essentially as an adjunct to the more morphological research. This, in fact, is the second shortcoming of the book - the emphasis on morphological aspects to the virtual exclusion of biochemical analysis. An enormous variety of cellular phenomena is described, but only in a few instances is there any discussion of the possible molecular basis for these phenomena.

But perhaps the most disappointing aspect of the book is its blandness. All of the information presented receives much the same degree of emphasis and enthusiasm. The authors rarely indicate a preference for one interpretation versus another, nor do they ever criticize omissions in the data, the lack of proper controls or internal inconsistencies. This is not a critical presentation of the data. It was rather disheartening to read through a book concerned with an area of such excitement and find only a compendium of facts. The failure to create any larger order out of the enormous amount of information available must be counted as a missed opportunity.

Daniel Rifkin is an Associate Professor of Cell Biology at New York University Medical School.

## Resources on a plate

Frederick J. Sawkins

Economic Geology and Geotectonics. Edited by D.H. Tarling. Pp.213. ISBN UK 0-632-00738-9/ISBN US 0-470-27145-0. (Blackwell Scientific/Halsted Press: 1981.) £15, \$54.95.

THE possible relationships of resources of various kinds to geotectonics is a subject that is attracting considerable attention nowadays, and this volume was apparently conceived with the object of providing an introduction to anyone — undergraduates, postgraduates and even seasoned researchers — with an interest in the field. Sadly, the book failed to live up to my initial expectations. It contains nine chapters by eight contributing authors, and opens with an introduction to the general theory and operation of plate tectonics. Although somewhat sparsely illustrated, this account provides a competent



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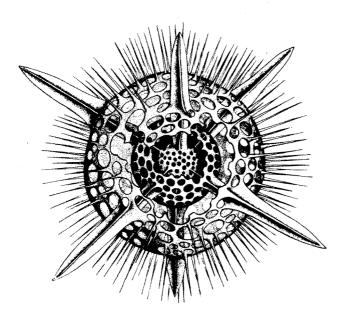
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## A close look at Springer MICROBIOLOGY



## The Prokaryotes

A Handbook on Habitats, Isolation and Identification of Bacteria

Editors: M. P. STARR, H. STOLP, H. G. TRÜPER, A. BALOWS, H. G. SCHLEGEL

1981. Approx. 433 figures. Approx. 2625 pages (In 2 parts, not available separately) Cloth DM 880-; approx. US \$ 409.70 ISBN 3-540-08871-7

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- identification their morphologies are meticulously defined and illustrated, and differentiated morphotypes within each group are compared and contrasted
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K. HORIKOSHI, T. AKIBA

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1981. Approx. 270 pages Cloth DM 98,-; approx. US \$ 45.70 ISBN 3-540-10924-2

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H. IIZUKA, A. NAITO

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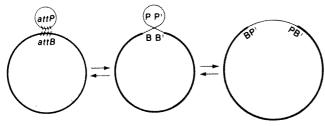
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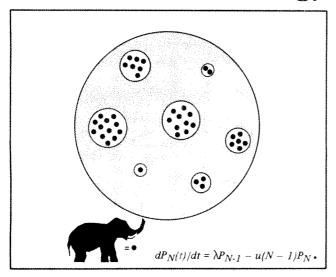
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The following three chapters deal with the formation, migration and accumulation of petroleum. Much of the material is a duplication of that to be found in any petroleum geology text. Plate tectonics is certainly dealt with in terms of basin development, but in view of the volume's title and its stated goals, this important subject deserved a more penetrating analysis and further fleshing-out with specific examples. Chapter 5 provides the reader with an overview of coal geology; again, much of the material is standard textbook stuff, and the chapter contains only half a page of general comments regarding possible plate tectonic controls of coal-generating environments.

The remainder of the book covers the relationships of metal deposits to plate tectonics. The chapter dealing with metal deposits formed at spreading ridges is well done and covers podiform chromite and Cyprus-type massive sulphide deposits. However, the association of ore deposits with subduction-related environments is covered in a mere seven pages of text, plus four illustrations; as a result of this brevity, many important types of deposits in subduction-related arcs are ignored, as are most of the more recent insights into arcmetal deposit relationships such as interarc rifting.

The contribution on the origins of ore deposits in sedimentary rocks is certainly of adequate length and contains a good deal of information, although the writing is somewhat laboured. I was also confused by the author's lack of conviction on certain points. For example, less than a page after a statement that the relationship between "secondary sedimentary ores and some sort of growth faulting is so common as to be almost a requirement", the suggestion that rifting is active during mineralization is questioned.

The final chapter considers the significance of palaeoclimatic factors on resources and offers some general conclusions. It touches briefly on a host of varied topics, but contains one or two startling misconceptions, for example the suggestion that the copper-sulphide deposits of Kennecott, Alaska, have a detrital orgin.

The volume contains 64 figures, many of them rather simple sketches, and only a single table. There are also a number of text citations missing in the references.

Overall, one is left with the impression of a somewhat hastily assembled volume which falls short of its claim to "provide an integrated introduction to the subject of economic geology".

Frederick J. Sawkins is a Professor in the Department of Geology and Geophysics at the University of Minnesota.

## Solving big problems on big computers

F. James

Computer Simulation Using Particles. By Roger W. Hockney and James W. Eastwood. Pp.540. ISBN 0-07-029108-X. (McGraw-Hill: 1981.) \$49.50, £27.50.

It is a pleasure, in these days of makeshift books on computing and simulation, to come across a thoroughly coherent work painstakingly prepared by recognized experts in the field.

The authors address themselves to one of the three general approaches to the study of systems (liquids, galaxies, plasmas and so on) governed by known differential equations. The other two approaches, finite elements and finite differences, are already well covered in the literature, whereas direct simulation by following constituent "particles" of the system, although in many ways more interesting mathematically, has largely been ignored except in research papers. To say that this book fills the gap is something of an understatement.

The orientation of the book is truly that of computational physics (or, as the authors prefer, computational science). It is not just a physics book with a chapter on FORTRAN at the end. Although the subject is problems arising in physics and related areas, suitability to computation is the overriding criterion for all of the material presented; no model or technique is suggested which has not been used in actual computations, more often than not by the authors themselves.

Considerable attention is paid even to programming methodology, including a rather complete account of the OLYMPUS programming system. Such a chapter could, of course, be grafted onto any book, but it finds a natural place in this work where the coverage of fundamental properties of algorithms includes not only their theoretical convergence rates, stability and such, but also actual computer time and storage requirements, arithmetic precision requirements and sometimes suitability to particular computer architectures. The usefulness of this aspect could have been enhanced still further by including a table of comparison of different computers' performance, since of the computers most often cited (IBM 7090, 360/91 and 360/195, CDC 6600 and 7600, ILLIACIV, CRAY-1) most are rare birds, technologically obsolescent or both, and, apart from the CRAY, are unlikely to be commonly available in a few years' time.

Physicists will appreciate the authors' basic approach, the mathematical notation

Volume III of *The Northwest European Pollen Flora* has been published by Elsevier Scientific. Containing detailed studies of the pollen morphology of a further eight families, the book costs Dfl.110, \$53.75.

and the vocabulary. The reader is encouraged to consider the problems in their physical context, not just as mathematical abstractions. The importance of physical insight in the choice of mathematical methods is brought out clearly. In addition, the interdisciplinary nature of the work should make it especially interesting, for example, to an astronomer who can see the relationships between problems arising in the study of the orbits of galaxies and in the design of transistors.

The style of the book is characterized by clarity and simplicity, the hallmarks of authors who have something of substance to say and possess a complete grasp of their subject. The quality of the printing, the pleasant layout of text and, especially, the numerous and helpful illustrations (many computer-produced, of course) all contribute towards making the book a real pleasure to read. It is a first-rate reference book which will be indispensable to workers in the field, and it will certainly find its place as a textbook for advanced courses in both computer science and physics, as well as intermediate fields.

Frederick James is a staff physicist in the Data Handling Division of CERN, and a past chairman of the Computational Physics Group of the European Physical Society.



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## $\overline{P} = s$ . exp (E/kT): common ground in thermal physics

G.F.J. Garlick

Analysis of Thermally Stimulated Processes. By R. Chen and Y. Kirsh. Pp.361. ISBN 0-08-022930-1. (Pergamon: 1981.) \$60, £25.

WHAT might there be in common among such apparently diverse experimental operations as the thermal stimulation of luminescence or current on the one hand, and thermogravimetry or differential thermal analysis on the other? Add to these other processes measured in, for example, thermal annealing, evolved gas analysis, thermoremanent magnetism, thermal desorption, dilatometry and thermally induced creep, and there is the subject matter of the present text.

In their preface, the authors themselves make the comment that the book could look like a "marriage of convenience". However, there is more connection between the effects than that they each involve a change in some measurable physical parameter when a material sample is warmed up. The more subtle factor common to them all is the theoretical expression for the probability of the effect, which is the familiar Arrhenius exponential function  $P = s. \exp(E/kT)$ , where s is often known as the "frequency constant", E is the energy needed to effect the change in parameter, k is Boltzmann's constant and T the absolute temperature. The major problems of developing theory to correlate with any of them are not about this function but about the kinetics which determine what happens later. For instance, will an escaping electron recombine with its original location to give, for example, luminescence, or will it be retrapped?

A large part of the book is taken up with these kinetic variations and the reader interested in thermogravimetry or differential thermal analysis may find that his interests are somewhat overwhelmed by extensive treatments of thermally stimulated luminescence and conductivity. The volume will certainly not replace the many treatises on thermogravimetry or differential thermal analysis and other specific techniques to which the users of the associated, sophisticated equipment now turn. However, there is always the possibility that by reading of the existence of a wider range of thermally stimulated effects ideas may be transferred between the disciplines. In this sense the text will be very useful.

With the major concern of the authors being thermally stimulated electronic processes in insulators and semiconductors, there is a fairly exhaustive coverage of the theory and practice of studying "deep" levels in such solids. There are, however, some omissions and some inaccuracies. On a historical note, more credit should have been given to

Urbach for his quantitative assessment of thermoluminescence in 1930 which preceded that of Randall and Wilkins by many years. On the matter of retrapping the authors do not, in drawing examples from the literature, bring out the stillpuzzling case of zinc sulphide phosphors, where luminescence and conductivity can be co-explored to determine detailed mechanisms. In 1948 Wilkins and I (Nature 161, 565-566) showed that retrapping of thermally released electrons was very small even when empty traps outnumbered empty recombination centres by more than 1000:1. In another instance, when reviewing photon-induced transfer of carriers between trapping states, the enormous effect found in infrared-stimulable zinc sulphides by myself and Mason (J. Electrochem. Soc. 96, 90-113; 1949) is not referred to. These are important examples because they indicate ways in which retrapping can be examined in an important group of high band gap solids.

In spite of the problems of using thermally stimulated processes to obtain

accurate values of trap energies and crosssections, the recent development of DLTS (deep level transient spectroscopy) as a steady state method of thermal stimulation would appear to offer a breakthrough for determination of these parameters in device semiconductors such as silicon and gallium arsenide. It is a pity that some of the elegant examples of trap distributions obtained by this method were not included in the text.

There is no doubt that this book will be attractive to particular groups of readers who, like myself, have specific interests in carrier trapping and related processes in insulators and semiconductors. It is well referenced and has a critical approach to the use and interpretation of thermal stimulation methods. Whether its attempt to coordinate analysis of the many different thermally stimulated effects will appeal to a wider readership is less sure.

G.F.J. Garlick is a consultant specializing in the fields of luminescence and electronic processes in semiconductors.

## Developments in theorists' views of ecology

Richard Law

Theoretical Ecology: Principles and Applications, 2nd Edn. Edited by Robert M. May. Pp.489. ISBN hbk 0-632-00768-0; ISBN pbk 0-632-00762-1. (Blackwell Scientific/Sinauer Associates: 1981.) Hbk £20, \$42.75; pbk £10.50, \$23.95.

THEORETICAL ecology is a fast growing subject due, in large measure, to the stimulus of R.M. May and his associates; it is pleasing, therefore, that they should take the time to produce a new edition of their standard work (first published in 1976).

While retaining the same overall theme of developing a theoretical perspective from which to view the living world, the new edition contains some substantial changes. Several chapters have been modified in the light of recent work - most notably that on patterns in multi-species communities, which now considers intriguing questions such as how many trophic levels should exist and how theories about community stability and complexity can be tested. Several other chapters have been rewritten by new authors; in particular, that on plant-herbivore systems has been improved by taking greater account of herbivory in the real world. However other chapters that would have benefited from revision remain unchanged. For example, "Island Biogeography and the Design of Nature Reserves" hardly does justice to the promise of its title, design principles for nature reserves being relegated to a few paragraphs at the end. No mention is made of recent work in this area and of the debate that has come to surround the whole subject.

There are two welcome additions to the book. The first is a survey of the new kind of population dynamics that is emerging from studies of plants, in which individuals, rather than growing to a fixed size, may continue to grow indefinitely by the production of modular units of construction. The second, dealing with the economics of biological resources, follows an elegant path from ecological theory to practical questions of optimal harvesting and the regulation of fisheries.

The book does not attempt to give a comprehensive coverage of theoretical ecology — for example, the reader will search in vain for an adequate account of the dynamics of populations with age distributions or of the effects of genetic variability in ecological parameters. But any shortcomings are greatly outweighed by its merits; it is a lucid and up-to-date introduction to the contributions of R.M. May and his associates to theoretical ecology, and it deserves to be widely read by all those with an interest in the subject.

Richard Law is a Lecturer in the Botany Department at Sheffield University.

## ATTOUT CEME TS

## **Awards**

Aaron Klug (co-director of the Division of Structural Studies of the Medical Research Council's Laboratory of Molecular Biology at Cambridge University) is the winner of Columbia University's 1981 Louisa Gross Horwitz Prize for outstanding research in biology or biochemistry.

**Dr Adolf Schallamach** is the recipient of the Charles Goodyear Medal for 1982 for his contribution to the science and technology of rubber.

**Dr Waldo L. Semon**, inventor and holder of the basic patents for polyvinyl chloride (PVC), has been selected as the 1981 "Inventor of the Year" by the New York Patent Law Association.

**Prof. J. Michael Bishop**, (University of California, San Francisco) has been presented with the first Association of American Medical Colleges Award for Distinguished Research in Biomedical Sciences.

Candidates of international stature are sought for the 1982 Lita Annenberg Hazen Awards for Excellence in Clinical Research. The Award Committee will select a physician-investigator or team, who will be awarded \$50,000 (tax-free). An additional \$50,000 will be provided for the support, for up to three years, of a Research Fellow or Fellows whom the Award winner will select as associates. Nominations until 15 February 1982: to Myrna Turkeiwitz, Office of the Dean, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, New York 10029, USA.

The World Academy of Art and Sciences invites anyone interested in the Social Implications of Advances in Microbiology to nominate persons whose work may merit the II Stuart Mudd Awards to: Prof. Román de Vicente, Consejo Superior de Investigaciones Científicas, Joaquín Costa 32, Madrid — 6, Spain.

The Beilby Awards are made to British investigators in recognition of original work, which has been carried out continuously over a period of years and should have involved scientific principles in any field related to chemical engineering, fuel technology or metallurgy. The Awards are intended to encourage men and women, normally under the age of 40. Applications by 31 December 1981 to: The Convener of the Administrators, Sir George Beilby Memorial Fund, The Royal Society of Chemistry, Burlington House, London W1, UK.

The Meldola Medal will be made in 1982 to the chemist who, being a British subject and under 30 years of age at 31 December 1981, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Society of Chemistry before 31 December 1981. Applications to: The President, The Royal Society of Chemistry, Burlington House, London W1, UK.

## **Appointments**

Dr Max Siegel, adjunct professor of psychology at Florida Atlantic University, has been elected president-elect of the American Psychological Association (APA), effective January 1982. He will become president in January 1983.

**Prof. I.L. Dillamore,** Professor of Metallurgy and Dean of the Faculty of Engineering at the University of Aston, Birmingham, has been appointed Director of Inco Limited's European Research and Development Centre in Birmingham.

Charles Alexander McDowell, has been appointed university professor of The University of British Columbia, in recognition of his contributions to chemical sciences and to the university.

**Barry Raleigh**, succeeds Neil D. Opdyke as director of Columbia University's Lamont-Doherty Geological Observatory.

## Meetings

1 December, Chemical Neurotransmission Yesterday and Today, London (Miss J. Holman, The Ciba Foundation, 41 Portland Place, London W1, UK).

1-2 December, An Understanding of Water Losses, London (The Institution of Water Engineers and Scientists, 6-8 Sackville St, Piccadilly, London W1, UK).

3 December, Electronics and the Future of Money, London (IEETE, 2 Savoy Hill, London WC2, UK).

7 December, **Digital Communications**, London (IEETE, 2 Savoy Hill, London WC2, UK).

8 December, Flood Control and Damage, London (Miss F. Spindlove, Oyez IBC Ltd, Norwich House, 11-13 Norwich St, London EC4, UK).

8 December, Transport of Membrane Proteins through the Golgi Complex, London (ICRF, Lincoln's Inn Fields, London WC2, UK).

10 December, GMP-Registration Interface, London (Dr T. Turner, Hoechst UK Ltd, Hoechst House, Salisbury Rd, Hounslow Middlesex, UK).

11 December, The Assessment of Lung Damage, Oxford (Mr S.R. Moores, AERE, Harwell, Oxon, UK).

14 December, Clinical Applications of Monoclonal Antibodies, Edinburgh (The Royal Society of Edinburgh, 22,24 George St, Edinburgh, UK).

14 December, The Structure and Function of Immunoglobulin Genes, London (Dr C. Spry, Royal Postgraduate Medical School, Du Cane Rd, Løndon W12, UK).

15 December, Some Newer Methods for the Production of Carbon-Carbon Bonds, London (Mr S. Langer, The Royal Society of Chemistry, Burlington House, Piccadilly, London W1, UK).

3-8 January 1982, AAAS National Meeting, Washington DC (AAAS-Dept R 1515 Massachusetts Ave, NW, Washington DC 20005, USA).

7 January, Space Exploration of Major Planets, London (IEETE, 2 Savoy Hill, London WC2, UK).

8 January, The Inaugral Meeting of the UK Molecular Graphics Group, Leeds (Dr A.J. Morffew, IBM UK Scientific Centre, St Clement St, Winchester, Hampshire, UK).

11 January, Dosimetry in Diagnostic Radiology, London (Mr D.W. Field, Hospital Physicists Association, 47 Belgrave Square, London SW1, UK).

13 January, Dry Etching, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

14 January, Waste Materials in Concrete, Slough (Cement and Concrete Association, Conference and Training Centre, Fulmer Grange, Fulmer, Slough, UK).

13-15 January, Immunoglobulin D: Structure and Function, New York (The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021, USA).

15 January, Fast Neutrons, London (The British Institute of Radiology, 36 Portland Place, London W1, UK).

27 January, Power Semiconductor Devices, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

12-13 February, Appropriate Medicine, London (London Medical Group, Tavistock House North, Tavistock Square, London WC1, UK).

17-19 February, Cell Proliferation, Cancer and Cancer Therapy, New York (The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021, USA).

18 February, Advances in Cardiovascular Imaging, London (The British Institute of Radiology, 36 Portland Place, London W1, UK).

19 February, The Future in Radiology, London (The British Institute of Radiology, 36 Portland Place, London W1, UK).

22-24 February, Effective Management Techniques for Engineers and Scientists, Amsterdam (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, Netherlands).

## Advertisement

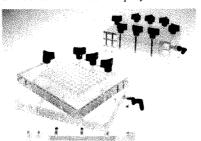
## BRL® PRODUCT REVIEW

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## DNA Polymerase I Lyphozyme™ (E. coli)

BRL announces the availability of the first in a line of freeze-dried modifying enzymes, DNA Polymerase I (E. coli). Because of their intrinsically labile character, both restriction endonucleases and DNA modifying enzymes have a finite lifetime, and require special shipping and storage conditions. For more than two years, BRL has made available lyophilized restriction enzymes (Lyphozyme'") and continues to expand the enzymes made available in this convenient, stable form. As the first freeze-dried modifying enzyme, DNA Polymerase I is an important addition to the line of products already available from BRL. Each lot of DNA Polymerase I is assayed for contaminating endo- and exonuclease activity and qualified for use in nick translation protocols.

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## HYBRI•DOT\*\*

BRL has designed a 96-well manifold that simplifies scale-up of the sensitive "dot hybridization" procedure for detection of specific DNA and RNA sequences. In this procedure, as little as one picogram of a specific nucleic acid sequence, bound to nitrocellulose as a small spot, can be detected using <sup>32</sup>P-labeled probe ( $\sim 10^8 \,$ dpm/ $\mu$ g). Dot hybridization has been used to detect viral DNA in lymphocytes and in serum.

The 3 mm diameter sample wells of the HYBRI•DOT™ system are arranged to precisely match the array of a standard 96-well microtitration plate. This simplifies and accelerates procedures such as the large-scale screening of bacterial colonies for recombinant plasmids.

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## AMV Reverse Transcriptase

BRL is pleased to make available reverse transcriptase (RT) from the avian myeloblastosis virus (AMV). BRL qualifies each lot of AMV RT for its ability to synthesize full-length cDNA copies of rabbit reticulocyte globin mRNA in the presence of an oligo (dT) primer. At least 80% of these transcripts must be full-length copies. BRL is the only commercial supplier to qualify AMV RT for transcription of natural mRNA's into cDNA's.

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## BRL® 3'End Labeling System

BRL is now pleased to make available the BRL® 3' End Labeling System. Using terminal deoxynucleotide transferase (TdT), BRL provides complete instructions and reagents for labeling DNA with either radioactive cordycepin triphosphate (supplied by the investigator) for subsequent Maxam-Gilbert sequencing reactions or with deoxynucleotides for tailing DNA for the purpose of cloning double stranded cDNA.

Along with complete instructions, the BRL® 3' End Labeling System contains TdT, deoxynucleotide triphosphates, buffers and a test DNA.

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## 5' Terminus Labeling System

The convenience of high quality tested reagents for labeling the 5' terminus of either DNA or RNA is now available from BRL. The BRL® 5' Terminus Labeling System is composed of T4 polynucleotide kinase, buffers and a protocol for either an exchange or forward reaction. Using the BRL® System, 5 x 10<sup>5</sup> cpm/pmol and 1.5 x 10<sup>6</sup> cpm/pmol can be incorporated into a 5' extended DNA terminus by exchange and direct reactions, respectively.

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## Lambda In Vitro Packaging System

BRL is now pleased to make available the Lambda In Vitro Packaging System. Based on the procedure developed by Enquist and Sternberg, the BRL® In Vitro Packaging System can be used in conjunction with λ1059 (BRL #5366), λgt WES•λBDNA (BRL #5267) or λ Charon 28 (BRL #5352).

With the BRL® In Vitro Packaging System, packaging efficiencies of up to  $4 \times 10^7$  pfu/ $\mu$ g wild type  $\lambda$  DNA are routinely obtained.

Circle No. 51 on Reader Inquiry Card.

## Lambda 1059

BRL now offers a new "phasmid" cloning vector,  $\lambda$  1059. This vector, developed by Jonathan Karn and coworkers allows for selection of recombinant molecules when plated on  $E.\ coli$  lysogenic for  $P_2$ . Enzymes which generate the 5'-tetranucleotide end 5'GATC-3' (Bam HI, Bgl II, Bcl I), are compatible for cloning into this vector. Particularly useful is the ability to clone DNA fragments produced by random digestions with Sau 3A which has a 4 base recognition sequence of 5'IGATC-3'. BRL qualifies each lot of  $\lambda$  1059 DNA for its ability to be packaged into  $\lambda$  particles under  $in\ vitro$  packaging conditions.

Circle No. 52 on Reader Inquiry Card.

## Vaccinia Virus Capping Enzyme

Bethesda Research Laboratories is the sole commercial source of Vaccinia Capping Enzyme, which has three major applications in molecular biology. First, capping enzyme has been used to map transcription initiation sites of Xenopus laevis, preribosomal RNA, fibroin mRNA of Bombyx mori, early vaccinia virus transcripts and early herpes virus transcripts. Second, RNAs of satellite necrosis and influenza virus have been labeled by capping enzyme prior to sequence determination. Finally, capping enzyme has been used to study the role of the cap structure in mRNA binding to ribosomes and translation.

Circle No. 53 on Reader Inquiry Card.

## PRODUCT REVIEW

## Neisseria testing

A positive control kit, N. Microtech, for confirmatory testing of various Neisseria species has been announced by University Micro Reference Laboratory. The kit contains 20 gels each of Neisseria gonorrhoeae, N. meningitidis, N. sicca, Branhamella catarrhalis and N. lactamicus. A gel is either streaked onto a plate or inoculated into the appropriate medium and incubated in a 4% CO<sub>2</sub> atmosphere. Applications include evaluation of medium for ability to support growth and analysis of standard methods of identification of clinical specimens.

Circle No. 100 on Reader Enquiry Card.

## Antibiotic susceptibility

A MANUAL now available from Mast Laboratories describes their range of antibiotic susceptibility testing products. Included are antibiotic susceptibility disks, agar dilution products, inoculators and other accessory products.

Circle No. 101 on Reader Enquiry Card.

## Spiral plater

Two new spiral plater instruments have been designed by Don Whitley to supercede the existing B250 models. The new model C retains the alternate deposition volumes of the B250 whilst reducing the plating time from 23 to 15 s for a 10-cm plate. An optional extra is a uniform cam which gives even deposition of sample across the plate. Developed for laboratories where standardized routine plating requires high operator productivity, model D has a single deposition volume of  $50\,\mu 1$  for 10-cm plates and an automatic plating cycle which has reduced plating time to 7 s.

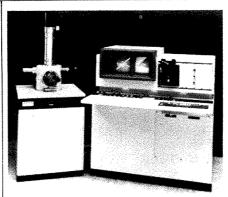
Circle No. 102 on Reader Enquiry Card.



## Viable counts

LUMAC instrumentation and reagents allow rapid microbial counting by the bioluminescence method. This is a rapid, accurate method for quantification of viable cells — a bacterial count is obtained in 30 s. It is useful where a quick result is desirable, for example, urine screening, contamination of food and dairy products, and water testing. It is also possible to count somatic cells, for example in tissue culture work.

Circle No. 103 on Reader Enquiry Card.



## **Automatic focus SEM**

A NEW, computer-controlled scanning electron microscope is available from Hitachi, Scientific Instruments. The S-520 provides resolutions of 60 Å, at magnifications from  $\times$  20 to  $\times$  200,000. Accelerating voltage range is 1-30 kV, selectable in 1 kV steps. A key feature of the S-520 is pushbutton-controlled automatic focus, which is achieved in 2 s, at magnifications up to  $\times$  50,000 and at working distances of 5-35 mm. An automated magnification system permits precise magnification settings in seconds and includes automatic brightness and contrast control, automatic 'quicklook' mode ( $\times 1,000$ ), a micron marker and one-button camera operation. All operating parameters are displayed automatically on the CRT, and a keyboard allows direct entry of other data. The standard S-520 stage has a 4-inch specimen chamber; a stage is available for semiconductor applications that handles 5-inch wafers and masks.

Circle No. 104 on Reader Enquiry Card.

## Instant photomicrography

An instant photomicrophy system, the SX-70, which produces high quality, detailed, full-colour microscopic images, has been introduced by Polaroid. The system has automatic exposure control and attaches easily to many microscopes. The SX-70 includes the new Polaroid SX-70 microscope adapter, a time-zero autofocus land camera model 2, and type 778 land film. The compact microscope adapter accepts folding single lens reflex SX-70 cameras and fits monocular and binocular microscopes or other optical instruments with eyepiece or phototubes of diameters between 24 and 40 mm. Adapter and camera electronics are linked to provide automatic exposure between 0.1 and 12.5 s. Circle No. 105 on Reader Enquiry Card.

PRODUCT REVIEW — The product review this week includes items for microbiology. The notes on these pages are prepared from information provided by the manufacturers. For more details use the reader enquiry card bound inside the journal. The next product review (26 November) deals with biochemicals.

## **Bacterial analysis**

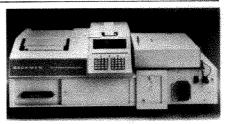
To detect bacterial contamination that may interfere with analyses, Millipore have produced the SPC sampler. This provides results equivalent to standard methods and the entire sampling procedure takes less than 2 min.

Circle No. 106 on Reader Enquiry Card.

## Media supplements

CULTURE media supplements in tablet form are now available from Mast Laboratories. Selectatabs are freeze-dried tablets containing one or more selective agents in an inert non-interfering carrier substance. On addition to the appropriate volume of medium, the Selectatab dissolves and a specific concentration(s) of the selective agent(s) is achieved. Selectatabs are available for the selective isolation of anaerobes, pathogenic Neisseria, pneumococci and Bordetella sp. Also available is a Selectatab for the prevention of swarming by Proteus sp.

Circle No. 107 on Reader Enquiry Card.



## Microplate analyser

As an accessory to the DU-8 spectrophotometer slab gel scanning system, Beckman Instruments has introduced the Microplate Analyzer to provide convenient, accusate analysis of microtitration plates. The Microplate Analyzer can be used in enzyme-linked immunoabsorbent assays and in the screening of monoclonal antibody production, quantitative determinations of antibodies and antigens, and bacterial growth studies. The system is useful in any kinetic, end point or microchemistry analyses.

Circle No. 108 on Reader Enquiry Card.

## Specimen handling

DANGERS associated with the taking and handling of swab specimens have been minimized by the development of Transwab, available from Medical Wire & Equipment. A new design of swab cap, used in all the units now supplied, has an elongated 'skirt' which completely encircles the top of the transtube, so making it almost impossible for the user's fingers to come into contact with any potentially infected part of either swab or tube. This eliminates the risk of cross-infection associated with the normal type of plug cap.

Circle No. 109 on Reader Enquiry Card.

## Anaerobic work station

An anaerobic work station which allows the operator to use bare hands whilst manipulating cultures at incubator temperatures has been developed by Don Whitley Scientific. Specimens can also be passed in and out of the cabinet through a special sleeve and glove port system without using the main microprocessorcontrolled interchange lock. The cabinet is fitted with automatic relative humidity control and external catalyst, atmospheric scrubber and dessicant canisters. The atmosphere is strictly controlled ensuring the earliest possible isolation of fastidious anaerobes. The cabinet accommodates 500 Petri dishes and allows ample space for inoculation, manipulation and examination of cultures.

Circle No.110 on Reader Enquiry Card.

## Agarose sampler kit

ON offer from Marine Colloids is a new agarose sampler kit. Included in the sampler are five of FMC Corporation's most popular agaroses covering almost all applications, including electrophoresis of proteins, nucleic acids and cells. One of these is SeaPlaque, a low-gelling temperature agarose used for preparative electrophoresis of nucleic acids and proteins, cell culture and cloning.

Circle No.111 on Reader Enquiry Card.

## Microfiltration

POLYCARBONATE and polyester capillary pore membranes are described in a new brochure from Nucleopore Corporation. These membranes alow high-resolution viewing against an unobtrusive two-dimensional background, advantageous in chemotaxis, exfoliate cytology, rheology, virology and other fields. Information on pore sizes, flow rates and applications are included in the brochure.

Circle No.112 on Reader Enquiry Card.

## **Fermenter**

A NEW fermentation vessel from Life Science Laboratories is available in 15 or 20l total volume, with the vessel, drive motor and all fluidic connections/fittings etc. mounted on a mobile trolley. The system is designed for in situ steam sterilization and can be operated at pressures of up to 2 bar g. The agitation system incorporates a double mechanical seal, which ensures sterility by maintaining positive pressure of sterile air in the seal housing. Air then enters the vessel by a rotating sparger. High oxygen transfer rates are possible with the 1.25 h.p. motor. A separate bench-standing cabinet contains compact instrumentation offering digital display of fermentation parameters, accurate control and computer-compatible output/control input circuitry.

Circle No.113 on Reader Enquiry Card.

## Dissolved oxygen meter

A portable dissolved oxygen/B.O.D. meter for use in wastewater, B.O.D. and other environmental monitoring applications has been developed by L.G. Nester. Featuring a range of 0-20 p.p.m. and a LCD readout, the model 8500 operates with either a submersible probe for wastewater pond and stream applications or a tapered B.O.D. probe for laboratory use. Both probes eliminate membrane changing, electrolyte replacement and the need for mechanical agitators or stirrers. Circle No.114 on Reader Enquiry Card.

## Streptococcal identification

It is now possible to identify the viridans streptococci and  $\beta$ -haemolytic organisms that either cross-react or give doubtful results with serological identification methods, by using a new design of API 20STREP, which includes 30 taxa on its data base. The tests are more easily interpreted and the centrifugation step required by the previous system has been eliminated. Circle No.115 on Reader Enquiry Card.

## Sampler loop

To avoid the hazards of splatter and aerosol spread, Hughes and Hughes Ltd. have produced a new sterile loop, made from polystyrene and sterilized by yirradiation. It performs the same function as a wire loop but incorporates the following features: calibration for semi-quantitative plating and counting, heel that allows easy spreading to give discrete colonies, an incorporated colony picker which avoids the need for a separate wire, and serrated section for easier disintegration of sputum or faeces.

Circle No. 116 on Reader Enquiry Card.

## Microbiology products

INCLUDED in the range of microbiology equipment from Griffin & George are autoclaves, sterilizers and associated equipment, culture bottles, Petri dishes, transfer chamber, incubators, refrigerators, colony counter and anaerobic jar. New to the range are Wipex disinfecting cloths which are impregnated with disinfectant and give complete disinfection on contact. Indicator stripes on the cloths show when the disinfectant is exhausted. They can be used for cleaning laboratory benches, transfer chambers, animal cages, washrooms, etc.

Circle No.117 on Reader Enquiry Card.

## **BIOLOGICAL SAFETY CABINET**

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## Anaerobe identification

A SYSTEM for simple and rapid presumptive identification of the most frequently encountered clinical anaerobes, has been developed by Flow Laboratories. The system consists of a broth tube, Anaerobe-tek broth, a plate of Anaerobetek, two reagents, Flow gelatinase and Flow indole II, and a computer code book. Anaerobe-tek broth serves as a cell suspension medium for the inoculation of the Anaerobe-tek plate, which consists of eleven peripheral wells and a central well containing solid media for the determination of 15 different biochemical parameters. Anaerobe-tek allows the following evaluations: hydrogen sulphide and indole production; activity of lecithinase, lipase and catalase and bile tolerance, among others. These parameters, together with Gram reaction, spore formation and cell morphology, permit numerical identification codes to be generated for a total of 18 test criteria.

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## Fluorescence microscopy

FLUOROCHROMATIC methods that require incident-light excitation are now commonly used in immunofluorescence microscopy, for example, in microbiology and phytopathology. Carl Zeiss Jena have produced for this purpose the Laboval 2a.fl incident-light fluorescence microscope, which has filter systems for excitation in the range 366-546 nm and apochromatic imaging optics of high aperture.

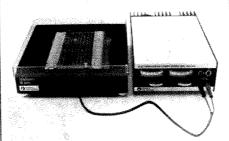
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## Microdispenser

ADJUSTABLE for volumes from 0.01 to 0.1 ml, the Micro repetitive dispenser from Tri-Continent can fit most bottles and serum vials from 5 to 500 ml. Two models are offered: one made of polypropylene, stainless steel and glass, the other of Teflon, platinum and glass. Applications are in radioimmunossay, micotitre assays,

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## **Immunoelectrophoresis**

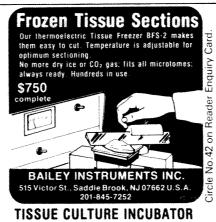
A NEW immunoelectrophoresis system for immunology and biomedical research has been introduced by Pharmacia Fine Chemicals. The system is designed for the fast running of all conventional forms of agarose gel electrophoresis and immunoelectrophoresis. All techniques are run on the flat-bed electrophoresis apparatus FBE-Immuno, which has an epoxy-resin construction resistant to coolant mixtures based on methanol, ethanol and ethylene glycol. The cooling plate itself has a large black surface area (152×250 mm) with a white printed pattern for easy visualization of immunoprecipitates, centering of the plates and direct sample application. An instruction manual is supplied with each FBE-Immuno apparatus.

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## Poured plates

POURED plates can be obtained from Gibco Europe. Each finished plate is screened and packed immediately, in units of 10, into nylon bags which allow the release of the resultant condensation, reducing considerably random contamination. A recent addition to the poured plate range is Modified New York City Medium.

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## Colony counter

AVAILABLE from Anderman & Company is a new pressure-sensitive counting system coupled to a digital counter. The Petri dish holder, which can accommodate dishes up to 90 mm in diameter, has been designed to allow 'edge counting' and colonies are evenly illuminated by diffused back lighting which eliminates operator fatigue. A new mechanical system ensures even pressure response over the entire surface of the Petri dish, and fast and accurate counting requires only slight pressure on the dish with any felt-tip pen or probe. An optional 114 mm diameter magnifier facilitates counting of densely populated cultures and fits conveniently on to the back of the colony counter body. A black background is supplied for 'side only' illumination, together with Wolffheugel grid plate and felt-tip pen.

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## Vortex mixer

PARTICULARLY useful for resuspending pellets after centrifugation, the new Monomixer, a vortex-type laboratory mixer from Sarstedt, Inc., has a variable speed control making it suitable for a variety of mixing applications. Thorough mixing in tubes and cuvettes up to 20 mm in diameter can be achieved easily with one hand by pressing the tube into the vortex cup.

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## Bacterial assay system

THE new BACTEC system from Laboratory Impex provides a means of detecting the presence of both aerobic and anaerobic bacteria in a wide range of materials. Two models are available, the semi-automated model 301B, or the model 460, which is fully automatic and can examine up to 60 culture vials per h or up to 480 vials per day. Results are automatically displayed on a built-in digital printer. Circle No. 125 on Reader Enquiry Card.

## Sterile filtration

A pressure-driven, disposable device for sterilization of tissue culture media, media additives, buffers and other aqueous solutions is available from Millipore. The Sterivex-GS filter unit will sterilize up to 41 of media or 11 of medium with 10% serum Because the Sterivex unit is pressuredriven, protein denaturation problems common with vacuum-operated devices are eliminated. A hydrophobic filter automatically provides venting for air which might enter the fluid path. Both the air vent filter and 0.22-µm low-extractable membrane filter are supported to withstand high pressures.

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## Protective gloves

CRYOGLOVES, which repel steam heat, are available in mid-arm and shoulder length. They are equally protective against ultracold atmospheres (for example, walk-in freezers, dry ice). Cryogloves, from Harris LoTemp, are water repellent and do not contain asbestos.

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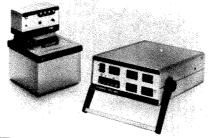
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## Temperature programmer

THE new Haake temperature programmer PG20, from Gallenkamp, can be used with Haake baths and circulators to give automatic time-linear temperature changes. All program parameters on the PG20 are set digitally, with rates variable from 0.01 to 9.99°C min-1.

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## $\beta$ -lactamase detection

AVAILABLE from Mast Laboratories, Intralactam is an acidimetric strip method for the rapid detection of  $\beta$ -lactamase. Originally designed to detect \(\beta\)-lactamase production in Haemophilus influenzae type B, it is suitable for use with gonococci and other Gram-negative bacteria. Results of such tests are available within 10 min. Detection of  $\beta$ -lactamase production in staphylococci is more difficult as the enzymes are produced only in small amounts, unless induced by the presence of a  $\beta$ -lactam antibiotic. Intralactam can detect staphylococcal \(\beta\)-lactamase by extending the reading time of the test. The use of a wet box prevents the strip from drying out and almost 100% of noninduced β-lactamase-producing staphylococci can be detected within 1-4 h. Circle No. 129 on Reader Enquiry Card.

## **Bacterial identification**

THE new system for identification of enteric and anaerobic organisms, Microbact from L.I.P., takes two forms, both using a microtitre plate. Microbact 12E consists of primary identification media for most isolates; Microbact 24E is a definitive identification set. The system is simple to set up and use and does not require a humidifying chamber. The user just has to peel back the tape covering the substrates, inoculate with a saline inoculum, replace the tape and incubate. After incubation the colour changes are noted with the aid of a chart. An 8-digit octal code system gives specific identification from the profile register.

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## Cryostar freezers

UNLIKE cascade freezers, the Queue Systems Cryostar freezers have only one compressor, and easily reach temperatures of ~75°C and ~100°C. The Cryostar line now includes a mechanically refrigerated -135°C cryogenic storage freezer, which can replace many expensive liquid nitrogen storage systems currently in use.

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## Chromatography

PRODUCTION scale chromatography columns for microbial enzyme technology are available from Wright Scientific. A new range covers diameters from 70 to 1,000 mm, in glass, stainless steel and acrylic. All the columns provide good resolution due to the spreading and collection characteristics of the end cells. Anti-jetting devices have been added to the larger columns.

Circle No. 132 on Reader Enquiry Card.

## **Automatic spreading**

THE Denley Autospreader has been modified - the new fully electronically controlled version allows fast accurate platingout for primary culture. The series of spreading loops used is automatically sterilized between plates, or before use, to prevent cross-contamination. Fail-safe logic shuts down the instrument if the loops fail to sterilize electrically.

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## **Incident light microscope**

An updated version of Reichert-Jung's MeF2 incident light microscope for routine photomicrography, the MeF2a, incorporates a fully automatic multi-camera system.

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## Cell disruptor

THE new model 350 Sonifier ultrasonic laboratory system from Branson Sonic Power Company is intended for ultrasonic disintegration and homogenization and is suitable for laboratory, pilot and production use. The power module of the 350 features a large front panel loading meter for ease of use. All units include a rear panel connection for the Sonifier temperature monitor TM-1 accessory that ensures accurate sonification exposure time while limiting the heat effects of the process. Typical applications for the Branson Sonifier include disruption of cells, bacteria, viruses and tissue.

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## Safety cabinet

THE laminar air flow design of the Bio-Safe microbiological safety cabinet prevents cross-contamination of cultures being used side by side within the cabinet. There are two filters, one which supplies sterile air to the work area, the other which filters the air being exhausted from the cabinet; 70% of the air is recirculated while 30% is discharged at each pass. These filters are 99.99% effective for particles of  $\geq 0.3 \,\mu\text{m}$ . Circle No.136 on Reader Enquiry Card.

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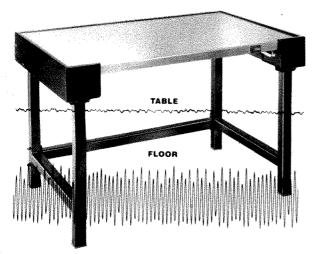
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Compiled by <u>Nature</u> from literally hundreds of sources, the wall chart offers data on actual dollars spent on science by government agencies, industry, foundations, universities, research and medical centers. The chart lists key U.S. government policy makers, with their addresses and phone numbers.

Most significantly, the chart shows the interlocking relationships within the science, engineering, and medical establishments.

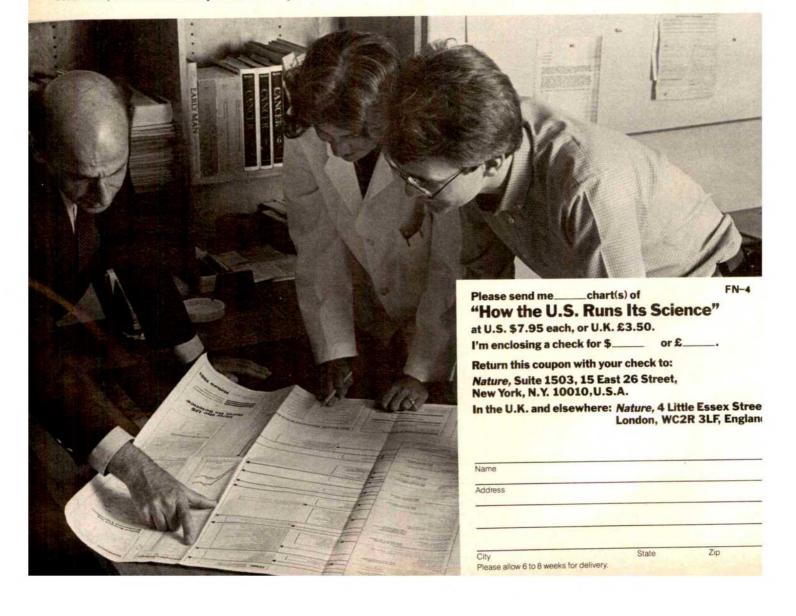
Designed as a fold-out that you can carry to meetings, or for hanging as a 2 x 3 ft. wall poster for permanent reference, the chart enables you to answer questions that

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Salary Scale: Dependant on class of degree and experience.

For application and job description, contact the District Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS. Telephone 01-737 0348 (24 hour Ansaphone) quoting reference no.

Closing date: December 4th 1981. (9906)A

## UNIVERSITY OF HONG KONG

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Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Appointments Unit, Secretary's Office, University of Hong Kong, Hong Kong.

The closing date for applications is (9911)A 25 January 1982.

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Interested individuals should submit résumés no later than December 1981 to Kathleen Acree, MD, Chief, Preventive Medical Services Branch, California Department of Health Services, 714 P Street, Sacremento, California 95814 (Telephone 916-445-3151). An equal opportunity/affirmative action employer.

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For further information contact: Prof. Dr med. Helmut Hahn, Institute für Medizinische Mikrobiologie der Freien Universität Berlin Hindenburgdamm 27, D-1000 Berlin 45, West Germany. (W498)A

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(9927)A

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The Rutherford Appleton Laboratory of the Science and Engineering Research Council require a Higher Scientific Officer to join a team engaged in the provision of the control centre, tracking station and science analysis facilities for the Infra-Red Astronomical Satellite (IRAS). This is a collaborative project involving the Netherlands, United States and the United Kingdom and the IRAS will be launched in the autumn of 1982.

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(9928)A

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For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW1006)A

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Closing date: Applications should reach the Swedish Natural Science Research Council, Box 6711, S-113 85 Stockholm, Sweden, no later than December 17th 1981.

For particulars apply to Mrs Natalie Lunin, Tel: 08-151580. (W505)A

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DEPARTMENT OF BIOLOGY

## CELL BIOLOGIST

The Biology Department invites applications for a faculty position in cell biology at the tenured or non-tenured level. Applicants working with a wide range of systems will be considered. Special attention will be given to applications in the area of development and differentiation, including cellular recognition, directed cell movements, and cell-cell interactions.

Our goal is to add an outstanding individual who will develop an active research program, attract graduate students and teach effectively. Interested candidates should send curriculum vitae, publication list, an outline of future research directions, and the names of three references to: Search Committee, c/o Malcolm Steinberg, Department of Biology, Princeton University, Princeton, New Jersey 08544, USA.

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Salary on the SPSO scale £14,329 to £17,418 per annum. Non-contributory superannuation scheme

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Interested candidates should submit curriculum vitae, bibliography, and names of three professional referees by 30 November 1981 to Dr Robert Friedman, Chairman, Department of Pathology, c/o Personnel/Manpower Division, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20014. The Uniformed Services University is an equal opportunity/affirmative action employer. (NW061)A

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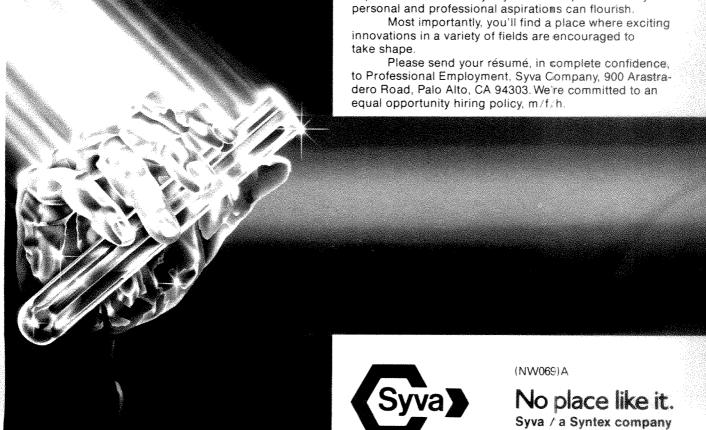
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stances and abused drugs in the body, with continued research and development efforts promising many more applications. And the formation of our new Instrument Division will ensure that Syva flourishes as a full-service organization, providing instruments, as well as reagents, service, and education to our customers.

Just as our accomplishments in the past are the result of innovative contributions from highly skilled professionals, Syva's continued growth and success depend on more of the same—more innovations from talented microbiologists, biochemists, organic chemists, technical service representatives and sales representatives. And since we're depending on whole-hearted determination to continue our success, our people depend on us for one of the most attractive work environments in the industry.

From a style of participatory management that encourages free and open discussion to the most advanced facilities in the business; from the beautiful San Francisco Bay Area's wealth of cultural and recreational resources to competitive salaries and benefits, when you explore a career with Syva you'll find a place where your personal and professional aspirations can flourish





The Swiss Federal Institute of Technology in Zurich invites applications for a

## Professorship in **Microbiology**

The duties of the professor include teaching in microbial metabolism and soil microbiology in the Faculties of Agriculture and Natural Science. Teaching courses should examine the connections between microbial physiology and ecological behaviour.

Detailed knowledge of microbial physiology and a broad understanding of the activities of micro-organisms in biogeochemical cycles are required. These should be the basis for research into nutrient cycling in agricultural and natural soils.

Applicants should have experience in university teaching and in directing research teams. Close collaboration with existing research teams in the field of microbiology is desired.

Applications with curriculum vitae and a list of publications should be submitted by December 31, 1981, to Professor H. Ursprung, President, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zurich.

(W499)A

## COWLES DISTINGUISHED **PROFESSORSHIP** IN BIOLOGY

Trinity University invites applications and seeks nominations for an outstanding scientist to fill the position of the Ruth C. and Andrew G. Cowles Distinguished Professorship in Biology. The Professorship is to augment a well established curriculum in biological sciences and to contribute significantly to the academic community.

The University seeks an individual of distinguished reputation in the biological sciences with an established record of excellence in teaching and scholarship, as well as a commitment to liberal arts education. The Cowles Professor will be selected from candidates with superior credentials in the biological sciences. Developmental biology and/or genetics are fields of particular interest.

Trinity University is an independent institution committed to excellence in the liberal arts and selected professional programs, including engineering. Present enrollment is 3,200, of which 2,600 are undergraduates. The Department of Biology has nine faculty members with the earned doctorate degree and 150 majors. The department offers a Bachelor of Arts, Bachelor of Science, and Master of Science degrees. Trinity University is located in the San Antonio metropolitan area which has a population of over one million.

Nominations and applications must be received by December 31, 1981, and should be mailed to:

Dr. Harold D. Murray, Chairman Department of Biology **Trinity University** San Antonio, Texas 78284

(NW082)A

An equal opportunity affirmative action employer

**CAMBRIDGE, MASSACHUSETTS** 

Positions are available for scientists at all levels who are skilled in the areas of:

Microbial Physiology Genetics **Cell Biology Nucleic Acid Biochemistry Recombinant DNA Technology** 

**Biochemistry Organic Chemistry** Enzymology **Protein Chemistry** 

Biogen is a biotechnology company with laboratories in the United States and Switzerland, developing commercial applications of molecular biology in the areas of agriculture, chemicals, pharmaceuticals and energy. We offer a challenging scientific career with the opportunity to interact with talented colleagues in a stimulating environment.

Research is currently underway in our laboratories in Cambridge and we are continuing to expand our staff and facilities during 1981-1982. seeking people in a variety of areas as we initiate new projects.

Biogen offers a competitive salary and benefits package

Reply to: Personnel Director, Biogen Inc., 50 Church Street, Cambridge, MA 02138. Please send your résumé and a list of three individuals whom we may contact for references on your behalf. All is an equal (NW071)A replies will be evaluated in confidence. Biogen opportunity employer.

NEUROPHYSIOLOGY / Respiratory Physiology: Research Associate Position, available immediately, for studies of neural and chemical control of ventilation. Doctorate required and postdoctoral experience preferred. Salary commensurate with experience. Curriculum vitae and names of three referees should be sent to: Walter M St John, PhD, Depart-ment of Physiology, Dartmouth Medical School, Hanover, NH 03755, USA. (Dartmouth College is an equal opportunity, affirmative action (male/female) employer.)
(NW055)A

> **UNIVERSITY OF MELBOURNE**

DEPARTMENT OF SURGERY Austin Hospital and

Repatriation General Hospital RESEARCH FELLOW GRADE 1

(Limited Tenure)

A Research Fellow is required to join a team based at the Austin Hospital studying the ontogeny, function and metabolism of peptides of the gut and the brain. The work involves research on foetal and adult sheep and in man.

Applicants should have a PhD (or near completion), background training in biochemistry, and experience in one of the following: physiology, pharmacology, or zoology. Previous studies in some of these areas would be an advantage; peptide chemistry including HPLC, peptide receptors, isolated organ perfusion.

Salary within the range of \$A17,083 — \$A19,570 per annum.

Enquiries to Dr A Shulkes or Professor K Hardy, Department of Surgery, Austin Hospital, Melbourne,

Further information, including details of application procedure and conditions of appointment, is available from the Staff Officer, University of Melbourne, Parkville, Victoria 3052, Australia. Applications referring to position number 546 A08 should be addressed to the Staff Officer, and close on 18 December 1981. (9912)A December 1981.

ASSISTANT/Associate Professor. Must have research interest in cellular and molecular aspects of hostparasite interaction. Postdoctoral experience in microbial physiology, molecular genetics, immunology, virology, or pathogenic organisms required. Teaching commitment in medical microbiology and graduate courses required. Closing date 15 February 1982. Send curriculum vitae, description of professional aspirations, and names of three referees to Dr Byron Burlingham, Department of Microbiology, School of Medicine, East Carolina University, Greenville, NC 27834. An Equal Opportunity through Affirmative Action Employer. (NW077)A

## CITY OF LONDON **POLYTECHNIC**

DEPARTMENT OF BIOLOGICAL SCIENCES Applications are invited for a

## RESEARCH ASSISTANT

to study the cell wall/outer membrane structure of Desulfovibrio with particular emphasis on the adhesion of the cells to metal surfaces. Applicants should have a good honours degree, preferably in Cell/Biochemistry, Chemistry or Microbiology. Experience of chromatographic and other analytical techniques will be an advantage. The post is available immediately and is tenable for two years and may be extended for a third year. The successful applicant will be expected to register for a higher degree with the

Salary (Researcher A) £4,386 pa in the first year of appointment, rising to £4,602 in the second year £4,818 in the third year. London Allowance of £759 pa is also paid.

Please apply in writing giving full curriculum vitae and the names and addresses of two referees to Mrs C. Gaylarde, Department of Biological Sciences, Old Castle Street, London, El 7NT. The closing date will be ten days from the appearance of this advertisement. (9893)A advertisement.

MEDICAL RESEARCH COUNCIL
CLINICAL AND POPULATION CYTOGENETICS UNIT

Cytogeneticist

Applications are invited for a short-term non-clinical scientist post (Grade II) in this MRC Unit, tenable for three years. Candidates of immediately post-doctoral or equivalent status will be preferred.

The appointee will work with a small group studying the chromosome changes that occur in patients with haematological disorders. Some experience in human cytogenetics would be an advantage but is not essential.

Remuneration will be at an appropriate point on the scales for University non-clinical academic staff.

Further information may be obtained from Mr. Alasdair Douglas, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Tel. No. 031-332 2471, with whom applications — including a full CV and the names and addresses of two professional referees should be lodged by 5th December, 1981.

## **RESEARCH POSITION**

(C1, Hochschulassistent) in antiinfective immunology available Applicants (PhD or MD) should have een interest and experience in antiinfective immunology (T cell cloning, receptor analysis, biochemistry of

This position allows ample time for research; in addition, participation in teaching lectures and courses in medical microbiology as well as work in the routine laboratory are obligatory. In the latter field, no special experience is required.

For further information contact: Prof. Dr med. Helmut Hahn, Institute für Medizinische Mikrobiologie der Freien Universität Berlin Hindenburgdamm 27, D-1000 Berlin 45, West Germany. (W497)A

## MANCHESTER AREA **HEALTH AUTHORITY** (TEACHING) CENTRAL DISTRICT **Tissue Typing Laboratory** Saint Mary's Hospital POST DOCTORAL WORKER

To join the North West Regional Immunogenetics Laboratory to undertake Research and Develop-ment work in the field of Renal Transplantation. The primary aim of the project is to improve transplant success by serological and cellular methods. Local facilities would allow access to many established and new

Interested persons with experience in Immunology, Genetic Markers, Tissue Typing or Cell Culture Techniques may discuss this vacancy with Dr Phillip Dyer (Tel 061-224 9633 ext 471).

Appointment will be according to qualifications at Senior Scientific
Officer or Scientific Officer level on
the Whitley Council PTA Scale. This
post is supported by the Manchester and North West Regional Kidney Research Association and is annually renewable.

Applications to Professor R Harris, Department of Medical Genetics, Saint Mary's Hospital, Hathersage Road, Manchester M13 (9919)A

An der Formal- und Naturwissenschaftlichen Fakultät der Universität Wien ist die Planstelle eines Ordentlichen Universitätsprofessors für

## SYSTEMATISCHE BOTANIK (mit Unterrichtsverpflichtung für Pharmazeuten)

zu besetzen.

Bewerbungen mit den üblichen Unterlagen (Lebenslauf und Schrif-Dekanat der Formal- und Schriftenverzeichnis) werden an das Dekanat der Formal- und Naturwissenshaftlichen Fakultät der Universität Wien, A-1010 Wien, Dr Karl Lueger-Ring 1, bis 31. Jänner 1982 erbeten (WS01) (W501)A 1982 erbeten.

## ASSISTANT OR ASSOCIATE **PROFESSOR AND** ASSISTANT OR ASSOCIATE **AGRONOMIST**

The Department of Agronomy and Range Science invites applications for an Assistant or Associate Professor/Assistant or Associate Agronomist position at the University of California/Agricultural Experiment Station, Davis, CA. Research should focus on integrative crop physiology and ecology as they relate to factors limiting performance of crop plant communities. The can-didate will be expected to develop an active research program pertaining to agronomic crops in California. Teaching duties will include one or two courses in either the undergraduate Agronomy or Plant Science programs and/or the Agronomy graduate program. A PhD in a suitable field is required. The level of appointment will be commensurate the candidates experience.

Send a statement of research interests, curriculum vitae, transcripts, list of publications, and names of three references by February 26, 1982 to: R L Travis, Search Committee Chair, Department of Agronomy and Range Science, University of California, Davis, CA

The University of California is an Equal Opportunity/Affirmative Action Employer. (NW079)A Action Employer.

## CSIRO AUSTRALIA Research Scientist/ Senior Research **Scientist**

\$A19,662 - \$A28,564

Division of Irrigation Research Griffith, New South Wales

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7500 employees - 2700 of whom are research and professional scientists located in Divisions and Sections throughout Australia.

Field: Root Zone Agronomy

General: Research of the Division is concerned with general problems of water management and with the efficient use of water and soils in irrigation agriculture and ecological consequences of inefficiencies. Fine textured soils of low permeability predominate in the irrigation areas of Australia and root zone limitations are often a major factor causing low yields in intensive systems of row crop production. A major goal of this Division is to develop management techniques for minimizing these limitations.

Duties: To undertake research on root zone factors causing low crop yields on irrigated fine textured soils. A major emphasis will be on the interrelationships of water and oxygen movement under systems of flood and controlled irrigation. The appointee will be required to oversee the development of a drainage lysimeter facility to study the impact of different techniques of surface irrigation, drainage and water table management on crop growth on the major irrigated soils of south east Australia. This work will provide the theoretical basis for a field program examining potential solutions to the low yield problem.

Qualifications: A PhD degree or equivalent in Agronomy or Soil Science and a proven capacity for independent original work at the postdoctoral level. Research experience with irrigation agronomy and soil drianage lysimeters would be an advantage.

enure: Indefinite with Superannuation

Applications: In writing stating full professional details, the names of at least two professional referees, quoting reference number A3017 should reach: The Acting Chief, Division of Irrigation Research, CSIRO, Private Mail Bag, GRIFFITH NSW 2680, AUSTRALIA, by 18 December 1981.

(9917)A

## AGRICULTURAL RESEARCH COUNCIL FOOD RESEARCH INSTITUTE

## **NUTRITION & FOOD QUALITY DIVISION NUTRITIONIST** (Scientific Officer)

The Food Research Institute has a vacancy for a Nutritionist in the Nutrition & Food Quality Division. This post is in the new group concerned with studies of the methodology of measuring food intake in man. The officer will be responsible for setting up and operating a nutrient information and data base. The post calls for a sound background in nutrition and an interest in the application of computers. The Institute shares computer facilities with the John Innes Institute and there is an active computer group with which this officer will interact.

Qualifications: A first or upper second class Honours degree in Nutrition or Food Science with experience of, and interest in, the application of computer techniques.

Salary: On a scale £5,176 - £6,964. Non-contributory superannuation

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting ref. 81/42. Closing date: 3rd December 1981. (9898)A

## ANGLO-AUSTRALIAN OBSERVATORY

## RESEARCH **ASTRONOMERS**

The Anglo-Australian Observatory comprises the 3.9 metre telescope near Coonabarabran N.S.W. and a laboratory at Epping near Sydney, Australia. There is a scientific staff of 6 astronomers nominated by the United Kingdom and Australia One position for each country becomes vacant between March and October 1982.

Candidates should have a strong background in physics or astrophysics. Appointments will be fixed terms of 1 to 5 years, with salaries in the range \$19,662 to \$38,921 per year, plus other allowances, fares etc. The successful applicants will have the choice of being based at the laboratory or the telescope. They will provide assistance to visiting astronomers, and contribute to the development, maintenance and general running of the AAT and its instrumentation. They will have opportunities for personal research and collaboration with Australian and UK astronomers.

Applications should include:

- Age, citizenship, country of residence and marital status.
- Qualifications and experience, including date of PhD.
- Astronomical research projects for which telescope time will be sought. Sufficient information is required to show that the proposals would be competitive in applications to a time assignment committee.
- Any instrument development project in which the applicant is particularly interested.
- When applicant would be available to take up appointment.
- Length of appointment envisaged.
- Names and addresses of three referees.

Applicants should arrange for the three references to be sent directly to the Director by the closing date.

Enquiries and applications should be addressed to the Director, Anglo-Australian Observatory, PO Box 296, Epping N.S.W. 2121 Australia, and applications copied to Mr I. L. Midson, Science and Engineering Research Council, PO Box 18, North Star Avenue, Swindon SN2 1ET, Wiltshire, England.

The closing date for applications is 4 January 1982. (9926)A



## PLANT TISSUE CULTURE

PHYTOGEN, a leading genetic engineering company applying molecular biology and plant tissue culture techniques for the improvement of agriculturally important crops, has an immediate opening at the level of SENIOR SCIENTIST in the area of PLANT TISSUE CULTURE. Duties will include the supervision of projects involving protoplast culture and regeneration. The successful candidate will have demonstrated the ability to conduct creative, independent research, minimum of two years postdoctoral experience is required.

PHYTOGEN is located in Southern California and offers a stimulating working environment. Applications accompanied by a curriculum vitae and a list of publications should be sent to: PHYTOGEN, 101 Waverly Drive,

Pasadena, California 91105.

(NW087)A

## Institute of Geological Sciences **GEOPHYSICIST**

The Institute has a vacancy for a geophysicist at Higher Scientific Officer/Senior Scientific Officer level in its Hydrogeology Unit based at Wallingford, Oxon. The successful candidate will be required to assist with overseas geothermal projects. Duties will include project identification, the preparation and management of prefeasibility projects and also research into the latest geothermal exploration techniques. Collaboration with consulting engineers in the design and preparation of bids for commercial projects will also be required.

Age and Qualifications: Applicants should normally be under 32 and have a 1st or 2nd class honours degree in an appropriate subject.

Salary: Higher Scientific Officer £6,530 - £8,589. Senior Scientific Officer £8,209 - £10,322

Grade of appointment and starting pay will depend on qualifications and relevant experience.

Starting salary may be above the minimum. Occupational pension scheme. The Staff of the Council are not Civil Servants but their pay and conditions of service are similar to those of scientists in the Civil

For an application form to be returned by 7 December 1981 please contact: Establishments (Recruitment) Section, Institute of Geological Sciences, Exhibition Road, London SW7 2DE. Please quote reference SO/HY/81/1.

NATURAL ENVIRONMENT RESEARCH COUNCIL

(9897)A

## MEDICAL RESEARCH COUNCIL MAMMALIAN **DEVELOPMENT UNIT**

Applications are invited for a

## SHORT-TERM NON-CLINICAL SCIENTIFIC POST

in this MRC unit, tenable for 3 to 5 years, to study early mammalian development. Candidates of immediately post-doctoral or equivalent status will be preferred; molecular or bio-chemical experience will be particularly welcome.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information and an application form may be obtained from The Secretary, MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, with whom applications, including a full CV and the names of two professional referees should be lodged by December 31 1981. (9920)A

## LOUGHBOROUGH UNIVERSITY OF **TECHNOLOGY** Polymer Physics Applications are invited for a three-year post as POST-DOCTORAL RESEARCH ASSISTANT

to take a leading part in an inviestigation of mechanical changes in PVC during polymerization. The award is a co-operative one between SERC and ICI Petrochemicals Plastics Division. A flair for physical measurements and instrumentation is required. Research experience in relevant fields is important. Starting salary £6,880. Reply to Dr Ray Wetton, Department of Chemistry. Loughborough, Leicestershire.

(9916)A

## **NEW YORK** MEDICAL COLLEGE **DEPARTMENT OF PHYSIOLOGY**

## POSTDOCTORAL RESEARCH **POSITION**

Available immediately under the sponsorship of Drs. Gabor Kaley and Edward J. Messina, Investigation of role of prostaglandins in regulation of microcriculation, hypertension and vascular reactivity. Background in microcirculation techniques, vascular smooth muscle work and small animal surgery preferred. Appointment is for one year. renewable for an additional year. Candidates should send CV, summary of research experience and names of three references to: Gabor Kaley, PhD, Professor and Chairman, Department of Physiology, New York Medical College, Valhalla, N.Y. 10595.

(NW072)A

## **GUY'S HOSPITAL** MEDICAL SCHOOL

## **POSTDOCTORAL** RESEARCH ASSISTANT

required as soon as possible until 30th November 1983 to join team investigating purine and pyrimidine metabolism in crippling inherited disorders. Preference given to Biochemistry graduate with some knowledge of the field and with experience in HPLC.

Salary for recently qualified PhD £6,070 plus £967 London Allowance.

Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting (9900)A Ref PM

## UNIVERSITY OF CAPE TOWN LECTURER IN BOTANY

Applications are invited for the above post. Preference will be given to candidates with research experience in either Ecophysiology or Marine Ecology/Phychology.

Appointment, depending on qualifications and experience will be made on the salary scale R10,995  $\times$  675 - R14,370  $\times$  810 - R19,230 per annum. In addition, a service bonus of nearly one month's salary is payable annually.

Staff benefits include 75% rebate on tuition fees for dependants at UCT, generous study leave privileges, housing subsidy subject to certain conditions, pension fund, medical aid and group life assurance schemes.

Applicants should submit a curriculum vitae, stating qualifications, teaching and research experience, publications, present salary, the date duty could be assumed and the names and addresses of three referees.

Further information may be obtained from the Registrar, (attention: Appointments Office), University of Cape Town, Rondebosch, 7700, by whom applications (quoting ref. no. . . .) must br received not later than 15th January 1982

not later than 15th January 1982.

The University's policy is not to discriminate in the appointment of staff on the grounds of sex, race or religion. Further information on the implementation of this policy is obtainable from the Registrar. (W506)A

## CORNWALL AND ISLES OF SCILLY AREA HEALTH AUTHORITY

Royal Cornwall Hospital (Treliske) Truro

## PRINCIPAL PHYSICIST

A new Department of Medical Physics, based at the Royal Cornwall Hospital (Treliske) Truro, is to be established in order to provide and expand the Medical Physics Services currently provided from Plymouth.

The Department will be developed in two phases, the main functions in Phase I being: (a) Radiation Protection; (b) Radiotherapy Physics; (c) Scientific Support Services in Nuclear Medicine; (d) Clinical Equipment Management in Electronics.

Premises available in Phase I consist of two laboratory/work-shops. Supporting staff will initially be two technicians MPT III) and a half-time secretary.

Plans for Phase II are not finalised but are in the Area Strategic plan. The functions will depend on clinical demand and on the effectiveness and enthusiasm of the Principal Physicist and his staff.

A physicist is now sought with the appropriate qualifications, and with substantial experience and proven success in most of the fields detailed above to set up the new Department.

The person appointed will initially be responsible to the Chief Physicist in Plymouth, but from the outset will have responsibility for internal management.

The facilities of the department at Plymouth will continue to be available as required.

Job description and application forms obtainable from the Personnel Officer, Cornwall and Isles of Scilly AHA, St. Clement Vean, Truro, Cornwall.

Full details are obtainable from Mr R.C.T. Buchan, Chief Physicist, Freedom Fields Hospital, Plymouth (752) 834267). Closing date: 4 weeks from the appearance of this notice.

(9894)A

## INSTITUTE OF ANIMAL PHYSIOLOGY

Babraham, Cambridge CB2 4AT

## RESEARCH SCIENTIST

required in the Physiology Department to undertake research into one of the following fields: hormonal control of gene expression; cell surface and membrane properties particularly of embryonic tissues; or the mode of action of gut peptides. Applications are invited from candidates interested in the endocrine control of reproduction, lactation or digestion. 1st or 2 (i) honours degree and at least two years' relevant postgraduate experience for HSO and four for SSO. A PhD would be preferred. Salary in Higher Scientific Officer Scale (£6,530 — £8,589) or Senior Scientific Officer Scale (£8,209 — £10,322) p.a. Noncontributory superannuation scheme.

Application forms and further details from the Secretary of the Institute, quoting reference PD7. Closing date 3 December 1981.

(9914)A

## **SCIENTIST**

There is a vacancy in the Medical Research Council Pneumoconiosis Unit for an X-ray Crystallographer to carry out research on the structure of mineral and man-made fibres of respirable size. The appointee will ioin the Physics Section which is engaged in development of methods to predict the potential health hazards of new fibrous materials and in investigation of the requirements for fibre carcinogenicity. The appointment will be for three years in the first instance and will be subject to MRC conditions for non-clinical scientific staff. The salary range is £5,505 — £8,845 (subject to review). Qualifications for entry are broadly similar to those for University

Applications should be sent to the Administrative Officer, MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, S. Glam., CF6 1XW. (9909)A

## ©SIRO AUSTRALIA Ceramics Scientist

\$A19.662 - \$A28,564

Division of Materials Science Advanced Materials Laboratory Fishermen's Bend, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7500 employees — 2700 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

Field: Electron Microscopy.

General: The Division has two laboratories, one located at the University of Melbourne (Catalysis and Surface Science) and the other at Fishermen's Bend. The appointee will be located at the Fishermen's Bend Laboratory where the main interests are in the mechanical properties of advanced ceramics and the electrical properties of ceramic electrolytes. There is wide interest in the Division's zirconia toughened ceramics.

**Duties:** The appointee will collaborate with a group studying the mechanical and thermomechanical properties of multiphase ceramics, including oxides, nitrides and carbides. Initially the appointee will work on sintering problems in the field of zirconia toughened ceramics.

Qualifications: A PhD degree or equivalent in an appropriate field, with demonstrated research ability and a thorough knowledge of Transmission Electron Microscopy, Scanning Electron Microscopy and Electron Analytical Techniques. A knowledge of sintering processes in ceramics and experience with ceramic powder characterisation would be desirable.

Tenure: 3 years. Superannuation benefits available.

Applications: In writing stating full personal and professional details, the names of at least two referees and quoting reference number A2728 should reach: The Officer-in-Charge, Advanced Materials Laboratory, Division of Materials Science, CSIRO, PO Box 4331, MELBOURNE VIC 3001 AUSTRALIA, by 18 December 1981.

## CELLTECH

## SEROLOGIST/HAEMATOLOGIST

Celltech is a new British Company, backed by the Government and City financial institutions, dedicated to the exploitation of discoveries in the field of biotechnology.

We are looking for a serologist or haematologist with about 3 years postodoctoral research experience in blood typing serology or related areas of immunohaematology to participate in a research programme designed to obtain, evaluate and develop new serological products. These products are most likely to be monoclonal antibodies and the successful candidate will be supported by our existing research effort in this field.

Salary will be in the range of £8,000 - £13,000, depending on age and experience.

Please send CV to the Personnel Department, Celltech Limited, 250 Bath Road, Slough, Berkshire St.1 4DY. Quoting ref. 120. (9915)A



## cetus

A pioneer in bioindustry since 1971, is currently seeking innovative professionals in the following areas:

- -Molecular genetics
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- Molecular virology
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- -Microbial physiology
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- -Protein chemistry
- -Plant cell genetics
- -Biochemical engineering
- -Process engineering
- -Instrumentation Design

We invite you to submit your resume in confidence to:

CETUS CORPORATION Box NKLH 600 Bancroff Way Berkeley, CA 94710 (415) 549-3300



(NW086)A

## BIOCHIMISTE/PHARMACOLOGUE — REGION PARISIENNE

Un des principaux groupes pharmaceutiques français du secteur privé recherche un biochimiste expérimenté orienté vers la pharmacologie moléculaire pour un poste de haut niveau, ayant une expérience d'au moins cinq ans dans l'industrie pharmaceutique, ou ayant dirigé une importante Unité de recherches à l'Université, de préférence dans le domaine cardiovasculaire, SNC ou sécrétion gastrique.

Ce poste conviendrait à un candidat pouvant prouver son expérience par une liste importante de publications.

Envoyer CV et liste des publications sous référence W502, c/o Nature, Macmillans Journals, 4 Little Essex Street, London WC2R 3LF.

Discrétion assurée.

(W502)

## THE UNIVERSITY OF SHEFFIELD

Applications are invited from men and women for the following post:

## Research Assistant Department of Psychology

To work on an SERC funded project investigating the functions of the superior colliculus in rats, especially in relation to the ascending dopamine systems. Training in relevant neurobiological techniques provided. Informal enquiries to Dr. Peter Redgrave (0742 78555 ext 6556). Tenable for 16 months in the first instance. Initial salary up to £5,675 a year on Range IB. Applicants should supply the names of two referees. Quote ref: R646/2/G.

Closing date for all the above post is 10 December 1981. Particulars from (candidates must quote ref. no.) and applications to the Registrar and Secretary (Staffing), the Registrar and Secretary (Staffing), the University, Sheffield S102TN. (9921)A

## FORESTRY COMMISSION Decay in Amenity Trees

## Higher Scientific Officer/ Senior Scientific Officer

required in the Pathology Branch of the Forestry Commission, Forest Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey to undertake research into decay problems associated with amenity trees. The research programme will consist of a continuation of current research on the treatment of pruning wounds

This appointment will be made under a contract between the Forestry Commission and the Department of the Environment which terminates on 31 December 1983.

Qualifications: 1st or 2nd class degree in biological sciences. Research experience on decay fungi in trees would be a considerable advantage.

<code>Salary:</code> Within the range for HSO or SSO - depending on qualifications and experience, ie £6,530 - £10,322.

Note: This is a Contract appointment up to 31 December 1983.

Applications forms and further details are obtainable from Mrs K. Burt, Forestry Commission, Personnel Branch, 231 Corstorphine Road, Edinburgh EH127AT. Tel: 031-334 0303 ext 406. Completed applications should be returned not later than 10th December 1981.

GUY'S HOSPITAL Medical School, Occupational Health Research Unit, Department of Community Medicine. Graduate or Postdoctoral Research Assistant required for two years to develop a portable electronic device for measuring back stress. Applicants should have a degree in Electronic Physics, preferably with bioengineering training or comparable experience; interest in physiological and occupational aspects of low back pain desirable. Salary in range £5,285 to £7,700 or £6,070 to £10,575 according to qualifications and experience, plus £967 London Allowance. Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, from whom further information may be obtained. Please quote Ref C M. Telephone enquiries to Department of Community Medicine (01-407 7600 ext 2157).

## PROFESSIONAL RESEARCH ASSISTANT IN CARBOHYDRATE CHEMISTRY

To participate in the purification and structural analysis of biologically interesting complex carbohydrates using modern instrumentation such as NMR, GC, GC-MS, LC and LC-MS. Applicants should have the equivalent of a BSc or MSc in Chemistry or Biochemistry. Starting salary (between \$12,000 and \$16,000) will depend on experience. The University of Colorado is an affirmative action/equal opportunity employer.

Applications should be sent to

Applications should be sent to Michael McNeil or Peter Albersheim, Department of Chemistry, University of Colorado, Campus Box 215, Boulder, Colorado 80309, and should be received by December 31, 1981. (NW089)A

## UNIVERSITY OF SUSSEX

Medical Research Council Cell Mutation Unit

## BIOCHEMIST/ MOLECULAR BIOLOGIST

Applications are invited for a Short-Term Non-Clinical post in this MRC Unit tenable for 3 to 5 years according to age and experience of the successful candidate. Candidates of immediately post-doctoral or equivalent status will be preferred, but well qualified candidates with less than 3 years postgraduate experience will also be considered.

The successful candidate will work in a team studying the mechanisms of DNA repair in human cells. Some enzymological experience is desirable and the work will involve the application of recombinant DNA technology to human DNA repair genes.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information and an application form may be obtained from Dr A R Lehmann (tel: 0273 606755, ext 236). Application forms plus a full cv and the names of two professional referees should be sent to Mrs M Bunn, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG by January 31st 1982. (9870)A

Please mention

## nature

when replying to these advertisements

## **MOLECULAR BIOLOGY**

The Microbiology Department at Case Western Reserve University invites applications for several tenure-track faculty positions. Although we encourage applications from candidates with strong credentials in any area of molecular biology, we are especially interested in applicants who propose to study regulation of gene expression, or molecular biological aspects of biology or immunology. Experience in recombinant DNA techniques is desirable. These appointments provide excellent opportunities to develop independent research and teaching programs in an expanding medical school department. Send curriculum vitae, a statement of research and teaching interests and the names of four references to Dr Fritz Rottman, Chairman, Department of Microbiology, Case Western Reserve University Cleveland, Ohio 44106. An Equal Opportunity/Affirmative Action Employer

(NW081)A

## **POST-DOCTORAL RESEARCH ASSOCIATES**

Positions are available immediately to work in the area of internalization and intracellular binding of yona dotropins and prostaglandins in corpora lutea. Candidates with experience in one or more of the following areas will be considered: subcellular fractionation; marker enzyme assays; prostaglandin biosynthesis and metabolism; membrane receptors; cell dispersions; cell and tissue culture. Send curriculum vitae and three letters of recommendation to: Dr. Ch. V. Rao, Dept. of OB/GYN, 436B MDR Bldg, University of Louisville, Louisville, KY. 40292. (NW075)A

## **UNIVERSITY OF** THE WEST INDIES

Trinidad

Applications are invited for the post of

## SENIOR LECTURER/ LECTURER IN **BIO-CHEMISTRY**

in the Department of Biological Sciences. Candidates should hold a good honours first degree in a bio-logical subject and postgraduate qualifications in Biochemistry. Research and teaching experience in plant biochemistry, microbiology or nutrition is desirable.

Salary Scale (to be reviewed): Senior Lecturer TT\$38,616 to TT\$49,092 pa; Lecturer TT\$29,784 to TT\$43,752 pa (£1 sterling = TT\$4.41). FSSU. Unfurnished accommodation or housing allowance. Family passages. Study and Travel Grant.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent as soon as possible to the Secretary, University of the West Indies, St Augustine, Trinidad.

Applicants resident in UK should also send 1 copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 90/91 Tottenham Court Road, London WIP ODT. Further details are available from either address. (9902)A

## POSTDOCTORAL POSITION

available January 1982 for 1 or 2 years. Research on intestinal absorption and metabolism of toxic metal ions. Biochemical and/or physiological training required and experience with absorption techniques desirable. Starting salary: about \$14,000. Send curriculum vitae and three letters of recommendation to: Dr. Robert H. Wasserman, Department of Physiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853. An Equal Opportunity/Affirmative (NW074)A Action Employer.

## **POSTDOCTORAL** RESEARCH POSITION

Two postdoctoral positions (post graduate research, 1-3 years) available January 1, 1982, for Plant Biochemist to study the Development of choloroplast thylakoids. Experience in organelle isolation, electrothoresis, lipid analysis, and/or enzymology preferred. Applications should be received by December 15, 1981. Salary \$15,936 to \$19,848.

Send curriculum vitae to: Dr N Terry, Department: Plant and Soil Biology, 108 Hilgard Hall, University of California, Berkeley, California 94720. The University of California is an Equal Opportunity/ Affirmative Action Employer. (NW085)A

## HIGH ALTITUDE **OBSERVATORY**

Visitor Appointments at the High Altitude Observatory are available for new and established PhD's for up to one year periods to carry out research in solar physics, solar terrestrial physics, and related subjects. Applicants should provide a curriculum vitae including education, work experience, publica-tions, the names of three scientists familiar with their work, and a statement of their research plans. Applications must be received by 15 January 1982, and they should be sent to: Visitor Committee, High Altitude Observatory, National Center for Atmospheric Research (NCAR), PO Box 3000, Boulder, Colorado 80307, NCAR is an Equal Opportunity/Affirmative Action (NW931)A Employer.

## ORGANIC CHEMIST

## **ANTIBIOTICS • ISOLATION** CHARACTERIZATION

SmithKline Corporation, a diversified multinational health care firm located in Philadelphia, Pennsylvania, is seeking an Organic Chemist for its suburban Philadelphia facility who:

- is able to isolate and characterize a variety of biologically active compounds from fermentation sources and other natural sources.
- can develop methods for detecting and isolating these
- can supervise a group in isolation and structure determination work.

Requirements include:

- PhD in organic chemistry with demonstrated post doctoral or industrial competence in isolation/characterization and
- structure determination.
  strong background in modern instrumental techniques including: HPLC, IR, UV, CMR, PMR, and MS. experience and desire to work at the bench with small and sensitive samples.
- ability and interest to participate in research planning with an interdisciplinary group of scientists.

SmithKline offers an excellent compensation and benefits package along with opportunity for personal and professional growth. Please forward your resume, in confidence, to: W.B. Flagg, Senior Employment Administrator, SMITHKLINE CORPORATION, 1572 Spring Garden Street, Philadelphia, PA 19101.

(NW070)A

We are an equal opportunity employer M/F/H/V

## **Rhizobiologists**

Agrigenetics Corporation is a broadly-based plant science company engaged in research and in the development, production conditioning and marketing of genetically-improved seed of more than 50 crop species and a variety of rhizobacterial inoculant

Opportunities now exist for several scientists (Ph.D. or MS.) to join the microbiology section of the Advanced Research Laboratory at Madison, Wisconsin in the area of Rhizobium genetics. Candidates familiar with the biochemistry and molecular genetics of Rhizobium in respect to symbiotic nitrogen fixation are invited to apply for new positions. Practical experience in working with Rhizobial plasmids and in recombinant DNA technology will be a major factor in the selection of candidates.

Successful applicants will join a group of more than 30 scientists working in various areas of plant molecular and cellular biology in a modern and attractive laboratory. Opportunities exist for interactions with additional Agrigenetics scientists at other locations, as well as the Corporation's applied divisions involved with legume species and in the fermentation and production of inoculants

Please send resume to the Director, Advanced Research Laboratory. Agrigenetics Corporation, 5649 East Buckeye Road, Madison, Wisconsin 53716



(NW073)A

Fisheries Research Laboratory, Lowestoft

Ministry of Agriculture, Fisheries and Food

## **Biologist/ Mathematician**

to assist with research and the application of mathematical modelling techniques for the assessment and management of exploited fish populations. Work will also include the preparataion, amendment and operation of computer programs and the supervision of junior staff.

Candidates, normally aged under 27, should have an honours degree equivalent in biology or mathematics.

Final year students will not be considered

Appointment as Scientific Officer £5,175-£6,960 with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 11 December 1981) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB or telephone Basingstoke (0256) 68551 (answering service operates outside

office hours. Please quote ref: SB/67/AD.

(9910)A

## <u>Scienti</u>

## Wanted writers for Indian Magazine

Those interested in promoting scientific matters in the third world are welcome to write features and reports on current scientific topics of general interest for the forthcoming fortnightly, Science Goodnews. Subscription price: £15 or \$20 including air postage. Write to: Ms. A. Sultana, 1 Chah Kanker, Victoria Street, Lucknow-226003, India. (W507)A

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The School stresses a multi-disciplinary and problem-oriented approach to undergraduates and postgraduate-teaching and research. It is committed to a number of broadly based field investigations of major issues.

## Lecturer — Ecology Salary range \$A19 821 - \$A26 037

The School has a vacancy for a lecturer with teaching and research experience in one or more of the following areas —:
Community, Population or Physiological Ecology of Vertebrates

The appointee will be required to teach in the Ecology and basic Biology components of the School's undergraduate programme. Duties will also include research and the supervision of postgraduate students in appropriate fields.

The appointee should be committed to inter-disciplinary teaching and research in a School community with a wide range of backgrounds and interests. An intending applicant will be expected to have a PhD degree. Demonstrated interests in such areas as wildlife management, conservation and the interactions between people and their environment would be advantageous. A first-hand knowledge of the Australian environment is preferred.

The position will be available from 1 July, 1982. It is offered as a continuing appointment with the expectation of tenure after the completion of an initial three year period.

Applications, including curriculum vitae and the names of three referees, and requests for further details about the position should be addressed to the School Administrator. School of Australian Environmental Studies, Griffith University,

NATHAN Q 4111, AUSTRALIA TELEPHONE — (07) 275 7427 The closing date for applications is Friday 1 January, 1982.

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## **FELLOWSHIPS**

## IMPERIAL CANCER RESEARCH FUND

**DEVELOPMENTAL GENETICS** LABORATORY (MILL HILL)

## **POSTDOCTORAL** RESEARCH FELLOWSHIP

Applications are invited for a three year postdoctoral appointment, starting September 1982, to work on the molecular genetics of pattern for-mation in *Drosophila*. The project involves the study of genes affecting the establishment of early embryonic pattern. The work makes use of recombinant DNA technology, genetic analysis and embryology, and experience of one of these techniques would be useful although not essential.

Salary range £7,700 to £9,750 plus Outer London Weighting (rate under review) with entry according to qualifications and length of post-doctoral experience.

For further information contact Dr D Ish-Horowicz at ICRF, Burtonhole Lane, Mill Hill, London NW7. Applications supported by full typescript CV, including date PhD obtained, list of publications and names and adresses of two referees, should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 by 31st December 1981 quoting reference

## MOLECULAR CELL **BIOLOGY PREDOCTORAL FELLOWSHIPS**

The Graduate Program in Molecular Cell Biology invites applications from highly qualified students interested in interdisciplinary PhD program. Major areas of emphasis include animal virology, bacterial and somatic cell genetics, cancer biology, cellular immunology and immunochemistry, connective tissue and protein biochemistry, infectious diseases and host defense, membrane biochemistry, nucleic acid synthesis, and research involving the cause and prevention of dental caries.

All students admitted to the program receive stipends of \$6,300 plus tuition and fees per year. After admission to candidacy the stipend is increased to \$7,000 per year.

Contact: Dr Roy Curtiss III, Director of Molecular Cell Biology Graduate Program, Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294. (NW078)E

## UNIVERSITY **OF SUSSEX** School of Biological Sciences

**POSTDOCTORAL** RESEARCH FELLOW

Applications are invited for a research fellowship to work on a project to isolate, clone and characterise the origins of DNA repliction from eukaryotic cells (see Expt. 1 Cell Res 129 (1980) 211-221; Nature, 284 (1980) 185-187). Experience in DNA recombinant techniques would be a great advantage. The project is for one year in the first instance. Salary on the scale for Research Fellow Grade 1A £6,070 to £10,575 pa.

Further particulars may be obtained from Professor Sydney Shall, Cell & Molecular Biology Laboratory, Biology Building, University of Sussex, Brighton, East Sussex BN1 9QG, to whom applications, including academic record, recent research work, a list of publications and the names of three referees, should be submitted to arrive no later than 31 January 1982. (9901)E

## **GRANTS** and **SCHOLARSHIPS**

## **UNIVERSITY OF** WESTERN AUSTRALIA Perth FORESTS DEPARTMENT **POSTGRADUATE SCHOLARSHIP**

Applications are invited from graduates with a first-class or upper division second-class honours degree in Botany, and Forestry or Agriculture graduates whose courses of study have extended over at least four years. The Scholarship, financed by the Forests Department of Western Australia, will be tenable for up to three years in the Department of Botany of the University, and is valued at \$A4,620 per annum; an additional dependants' allowance of \$A2,220.40 for wife, and \$A520 per child is available per annum.

The scholar will be required to undertake research for a PhD degree in one of three areas: (a) the biology of short-lived legumes which occur after fire in the jarrah forest, (b) the biology of an understorey tree, Banksia; (c) analysing reasons for the poor survival of Pinus seedlings after planting in old plantations. Other possible projects in forest ecology may be approved in consultation with the Department of Botany and the Forests Department of Western Australia.

Further information is available from the Head of the Department of Botany.

Applications in triplicate stating personal particulars, academic record, research experience, and other relevant information, should reach the Registrar, The University of Western Australia, Nedlands, Western Australia 6009, by 18 December 1981. Candidates should request two academic referees to write immediately to the Registrar.

(9913)H

continued on page xlix

## CONFERENCES and COURSES

## **EMBO PRACTICAL COURSE**

## **Automated Chemical and Enzymic** Gene Synthesis

Department of Biochemistry Technische Hochschule Darmstadt

## MARCH 21st TO APRIL 3rd, 1982

The teaching staff will include:
J.H. van Boom (Leiden), M.H. Caruthers (Colorado), H.J. Fritz (Köln), M.J. Gait (Cambridge, UK), H.G. Gassen (Darmstadt), W. Hillen (Darmstadt), K. Itakura (City of Hope, LA), H. Kossel (Freiburg), H. Köster (Hamburg), K.E. Norris (Bagsvaerd), E. Ohtsuka (Osaka), H. Schott (Tübingen), H. Selinger (Ulm), O.C. Uhlenbeck (Urbana), E.L. Winnacker (München) and others.

The programme will consist of practical work, lectures and seminars on

- solid-phase chemical synthesis of oligodeoxynucleotides
- phosphotriester method as related to the phosphoroamidite procedure
- automated oligonucleotide synthesis
- oligonucleotides from DNA fragmentation
- enzymic ligation of oligonucleotides
- recombination of RNA
- survey of oligonucleotide separation and analysis
- ligation of synthetic genes (promotors) to plasmid vectors

A maximum of 15 students will be accepted. Applications should include a short curriculum vitae and a description of present research interets. The registration fee for the practical course is 200, DM, which does not include food and lodging. A small number of grants in aid to cover partially board and lodging expenses may be granted to participants who are unable to obtain funds from other sources.

The weekend MARCH 27/28th will be kept free from practical work to allow the participation in a WORKSHOP entitled

## **Prospects of Automation in** Gene Synthesis

Informal application is sufficient for the workshop. The number of participants will be limited to 100. The registration fee is 60, DM.

## THE CLOSING DATE FOR APPLICATIONS IS JANUARY 15th.

Those accepted will be notified not later than January 31st, 1982. Applications should be sent to Dr H.G. Gassen, T H Darmstadt, Institut für Organische Chemie und Biochemie, Petersenstrasse 22, D-6100 Darmstadt. Phone: 06151/163657, Telex: THD 419579 (W504)C

## 3rd INTERNATIONAL **CONFERENCE ON BIOLOGY** OF VERTEBRATE AND **INVERTEBRATE SKIN**

of European Society for Comparative Skin Biology, in Krakow on 6-10 September, 1982. Details from Prof. M. Jakubowski, Dept. of Comparative Anatomy, Jagellonian University, ul. Karasia Krakow, Poland. (9895)C

## **GRANTS** and **SCHOLARSHIPS**

continued from page xlviii

## UNIVERSITY OF LONDON

## **GRANTS FOR RESEARCH**

Applications are invited from nembers of the University and eachers in Schools of the University or grants from the Central Research fund to assist specific projects of esearch and for the provision of pecial materials and apparatus. Grants are not made for normal naintenance.

Applications must next be received not later than 8 December 1981. Forms of application and further articulars may be obtained from the Secretary to the Central Research Fund, University of London, Senate House, Malet Street, WCIE 7HU. (9903)H

## SPECIAL FEBS MEETING ON **CELL FUNCTION AND DIFFERENTIATION**

April 25-29, 1982, Athens, Greece Information: Secretariat, NRC Demokritos, Dept. of Biology, Aghia Paraskevi, Attikis, Greece. Tel: 651-3111 ext 527. Telex: 21-6199.

(\A/482)C

## **FELLOWSHIPS**

continued from page xlviii

## FRIEDRICH MIESCHER-INSTITUT Basel **POSTDOCTORAL FELLOWSHIP**

is available from the beginning of 1982 (or later). The general topic is the structure, function and regulation of eukaryotic genes by steroid hormones. Emphasis is on the isolation, cloning, characterization, in vitro construction of mutants, in vitro and in vivo expression of cloned genes. Candidates should be experienced in the techniques of nucleic acid biochemistry and cloning. Applications (with curri-culum vitae) should be sent to Dr J-P Jost, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland.

NATO Advanced Study Institute Course on:

## GENETIC ENGINEERING IN EUKARYOTES

July 26 through August 6, 1982 **Washington State University** Pullman, Washington

Topics will include DNA transformation in fungi, mammalian cells and plant cells. Emphasis on development of vectors for gene transfer and expression in eukaryotes. Selection and biochemical characterization of eukaryotic mutants useful for transformation experiments. A proceedings will be published.

Participants will be limited to 85 and should be at least of postdoctoral level. There are a limited number of fellowships available.

Advisory Committee: E. C. Cocking, University of Nottingham, UK; O. W. McBride, NIH, Bethesda, MD, USA; E. W. Nester, University of Washington, Seattle, WA, USA; and J. Schell, University of Ghent, Belgium.

Organizing Committee: P. F. Lurquin, A. Kleinhofs, and W. A. Becker, Washington State University, Pullman, WA, USA.

Send inquiries to: NATO Advanced Study Institute, Conference Office, 323 Agricultural Sciences Building, Washington State University Pullman, Washington 99164.

(NW/076)C



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The course is open to medical, dental and veterinary practioners, graduates in science or pharmacy or other suitably qualified students. Candidates will normally be required to have at least one year's practical experience of Medical Microbiology.

Exemption from the Primary Examination for the MRC Path may be granted to suitably qualified British subjects resident in the United Kingdom taking the full-time course or the part-time course by the accumulation of credits. Closing date for applications 1 March 1982.

Further particulars from the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E7HT. (9896)C

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For further details and application form please contact: Dr M J Daly, Medical Division, Glaxo Group Research Ltd., Ware, Herts SG12 0DJ. Telephone No. 0920 3232.

## **SYMPOSIUM**

## "BIOMEDICAL RESEARCH: IMPORTANCE OF LABORATORY ANIMAL GENETICS, HEALTH, AND ENVIRONMENT"

The Fifth Charles River International Symposium will be held at the Sheraton Airport Frankfurt, Frankfurt, West Germany on March 9 & 10, 1982. Sponsored by the Charles River Foundation, the symposium will examine the importance of the laboratory animal to research and the researcher. For program and registration information:

Deutsches Reiseburo
Der Congress Eschersheimer Landstr. 25-27
6000 Frankfurt Am Main
West Germany
Attention: Mrs. Rockmann

(NW029)M

(9923)M

## **STUDENTSHIPS**

## UNIVERSITY OF ABERDEEN DEPARTMENT OF ANATOMY

BRITISH DIABETIC ASSOCIATION STUDENTSHIP

Applications are invited from suitably qualified candidates for a PhD Studentship provided by the British Diabetic Association for the study of peripheral nerve structure in experimental diabetic rats and genetically diabetic mice. The research will be conducted in the Department of Anatomy. The Studentship is tenable for three years from January 1982.

Further information from, and applications to, Dr A K Sharma, Department of Anatomy, University of Aberdeen, Marischal College, Aberdeen AB9 1AS (Telephone 0224 40241, Ext 237 or 233). Applications must be accompanied by the names of two references.

## **ANNOUNCEMENT AND CALL FOR PAPERS**

International Symposium on the Biomedical Effects of Ozone and Related Photochemical Oxidants March 14-18, Pinehurst, NC

Objective:

Ozone and related oxidants are a problem of increasing concern because of their potential for effects on human health. The objective of this symposium is to update the database on the biomedical effects of ozone and related oxidants of photochemical smog.

Symposium Program:

The program will contain seven sessions with up to five invited presentations and discussion by an invited panel. Volunteer contributions will be presented in poster sessions. The following topics will be included: atmospheric chemistry; biochemical and morphological responses and effects on pulmonary function in experimental animals and humans; effects of chronic exposures; post-exposure susceptibility to infection; and modifying factors.

Symposium co-chairmen are M.G. Mustafa and R.S. Bhatnagar. The session chairmen, listed alphabetically, are: A.P. Altschuller, R.S. Bhatnagar, D.L. Dungsworth, M.J. Evans, L.J. Folinsbee, R. Frank, D.E. Gardner, B.D. Goldstein, E. Goldstein, J.D.Hackney, J. Mead, M.A. Mehiman, D.B. Menzel, J. O'Neil, R.P. Sherwin and D.F. Tierney.

Symposium proceedings will be published incorporating peer-reviewed manuscripts.

Closing Date for Papers:

January 1, 1982.

For submitting abstracts and for registration and program information contact:

Institute for Biomedical and Environmental Studies, Inc. 2040 Pioneer Court, Suite 15, San Mateo, CA 94403
Telephone (415) 574-0615 or (213) 825-1153 (Dr Mustafa)
(NW084)M

## THE QUEEN'S UNIVERSITY OF BELFAST

## SERC RESEARCH STUDENTSHIPS 1982

The Science and Engineering Research Council is likely to offer to suitable candidates a limited number of research studentships for 1982 tenable in the Faculties of Science or Engineering; the former includes the Department of Archaeology, Applied Mathematics and Theoretical Physics, Biochemistry, Botany, Chemistry, Computer Science, Genetics, History and Philosophy of Science, Microbiology, Pharmacy, Psychology, Pure Mathematics, Pure and Applied Physics, Statistics and Operational Research, and Zoology; the latter, Engineering Mathematics, Aeronautical, Civil, Mechanical and Industrial, Electrical and Electronic, and Chemical Engineering Departments.

The value of these awards will be in accordance with SERC scales and students graduating in 1982 in Great Britain (and who are not resident in Northern Ireland) are invited to write stating their particular field of interest to the appropriate Dean, The Queen's University of Belfast, Belfast BT7 1NN, by *I December 1981*, or as soon as possible thereafter. (9899)F

## UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGY
AND PHARMACOLOGY

## POSTGRADUATE STUDENI HUMAN MUSCLE PHYSIOLOGY

Applications are invited for thi studentship tenable for 2 years in th first instance. The work concerns th physiology of elderly human muscl and will be supervised by Professo P H Fentem and Dr C T M Davies The Conditions of the studentship will the those applied by the Researci Councils.

Applicants should send thei curriculum vitae and names of academic referees to Professor P F Fentem, Department of Physiolog and Pharmacology, Medical School Queen's Medical Centre, Notting ham NG7 2UH as soon as possible.

(9925)F

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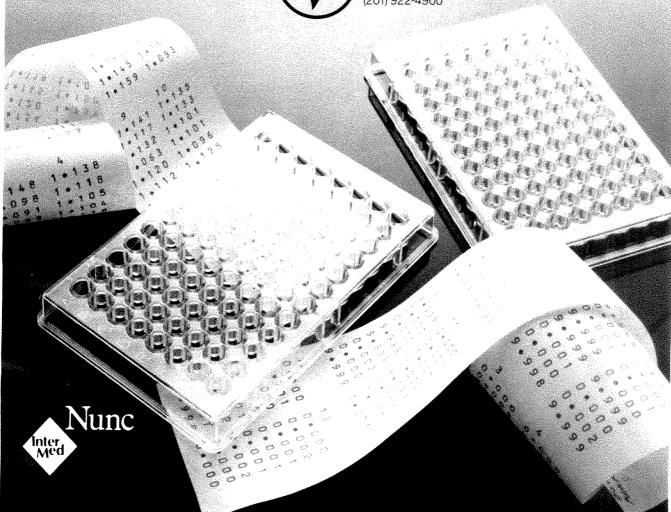
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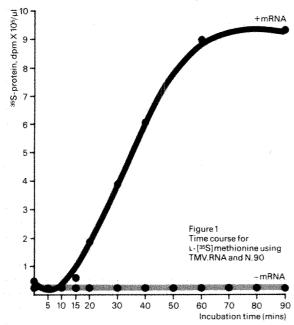
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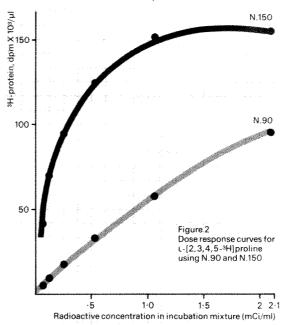
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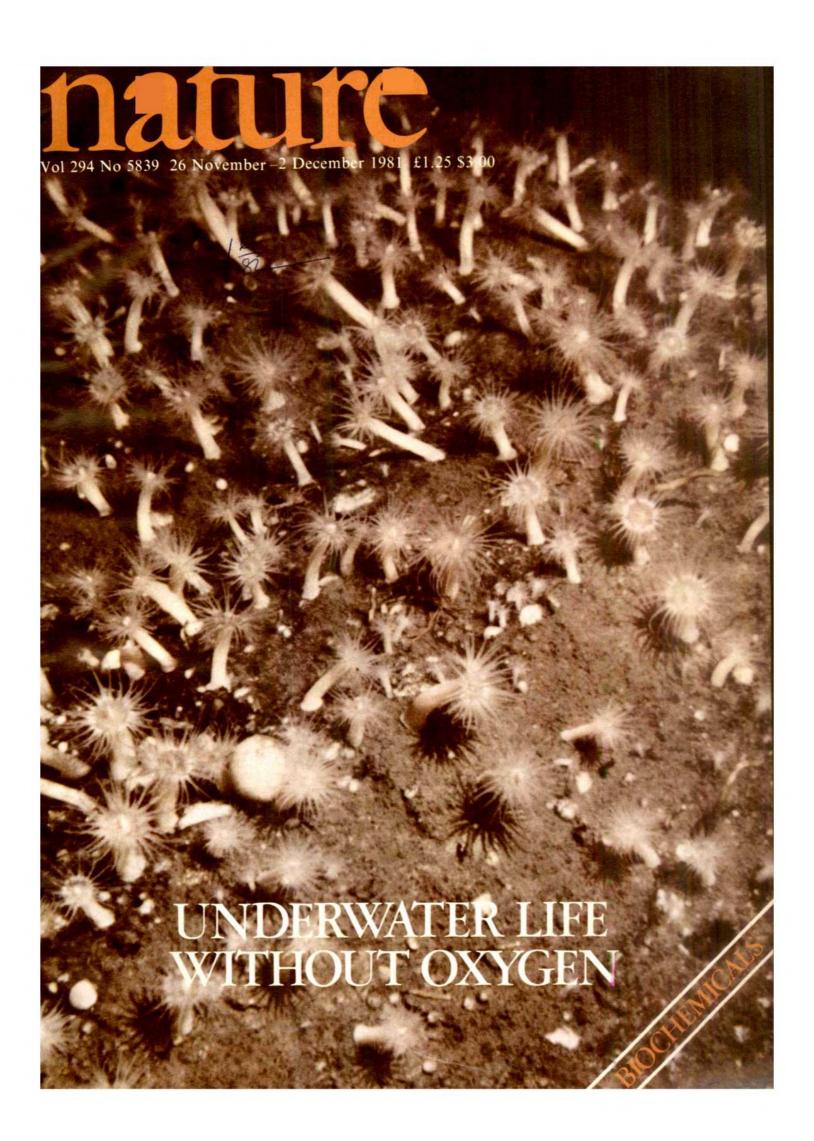
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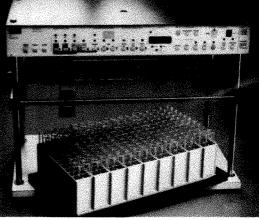
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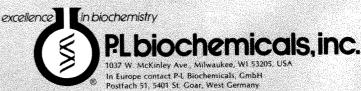
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nings to do today E

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2:00 Lunch with Lydia tons!

3:30 hydrolyze 8:30 sequence

3:30 hydrolyze 8:30 sequence

mer primer primer A 3'

4:00 make 16-mer primer primer A 3'

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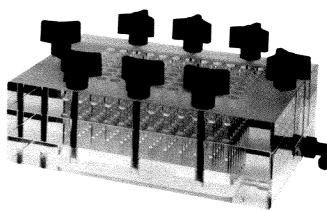


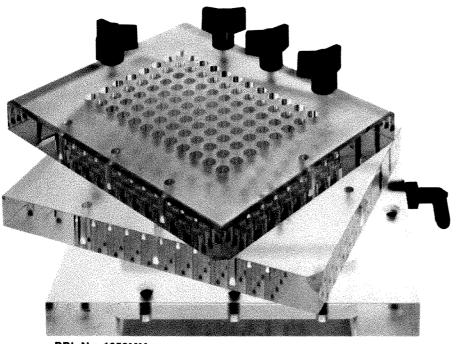
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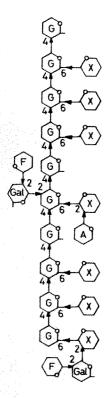
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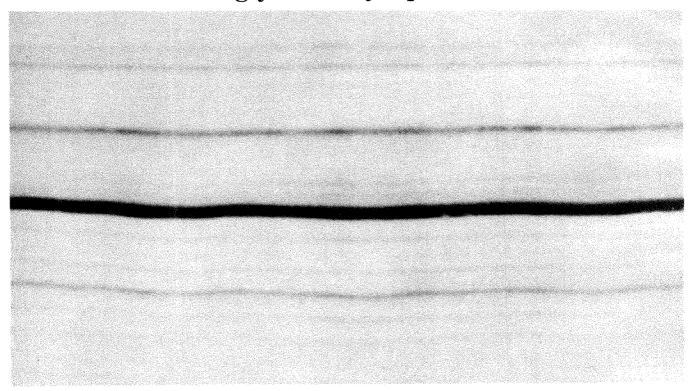
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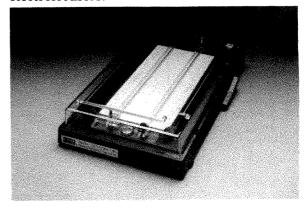
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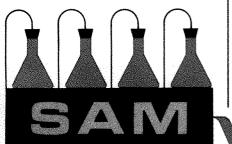
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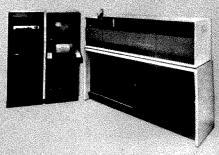
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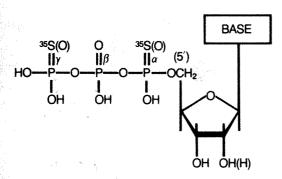


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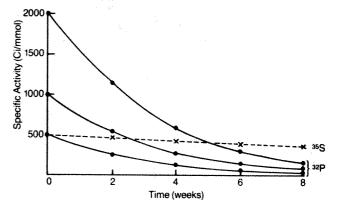


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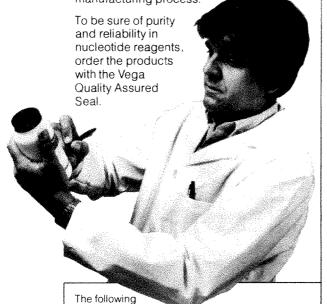
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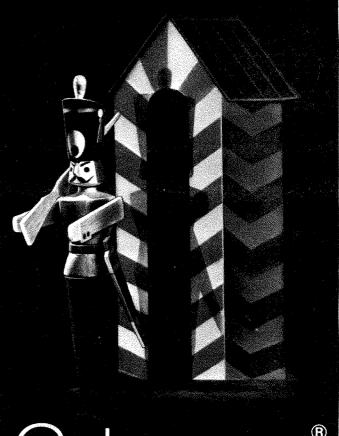


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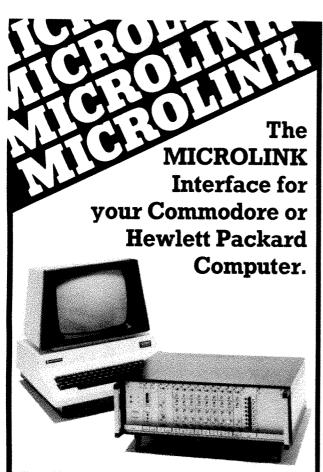
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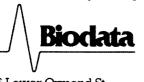
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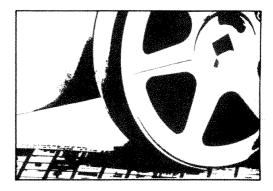
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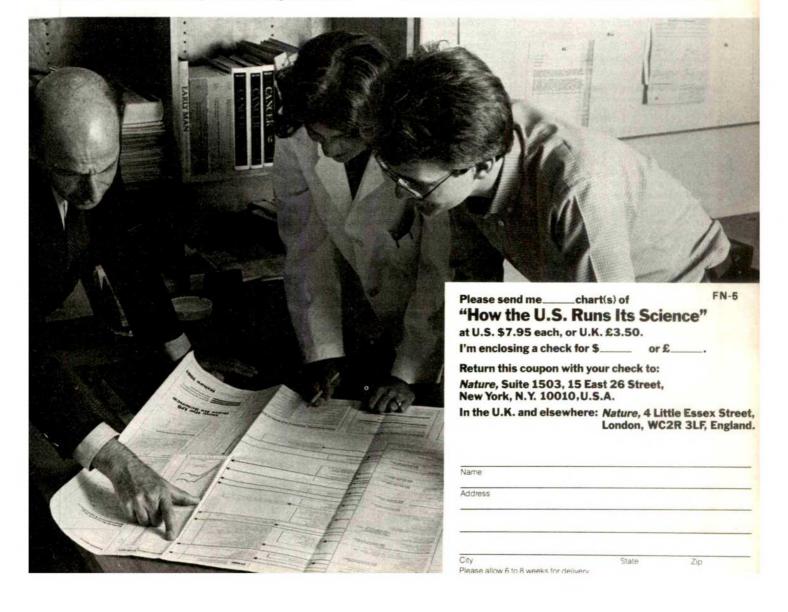
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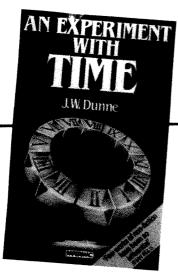
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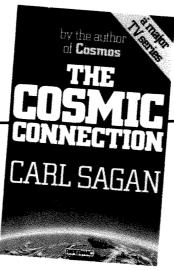
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#### 26 November 1981

#### Threats for British academic research

Research councils in the United Kingdom have recently been spared the axe. Is it now about to fall?

Mrs Margaret Thatcher, the British Prime Minister, was proudly telling the Parliamentary and Scientific Committee a year ago that the British government's spending on research had been "protected" from the round of government economies then coming into effect. Will she be able to make the same boast when the government's budget for 1982–83 has been completed? For the past several months, the research councils have been as much concerned with helping British universities out of trouble as with their own affairs. Now, with financial planning for 1982–83 under way, they are alarmed that, next time, the Treasury's finger will point at them.

The research councils have become vulnerable for several reasons, but chiefly because the British government's economic policies have failed. Neither the government nor its supporters can have imagined that it would be embarking on its fourth year in office (next spring) with yet another deflationary budget. By then, the assumption was, the benefits of financial prudence would be sweeping through the British economy. In practice, however, the government failed to implement the controversial but at least novel monetarist policies to which it was committed with anything like the vigour necessary to give them a sporting chance. So the chances are high that the British Treasury will need further reductions of public expenditure in the spring. The research councils are certain to catch its eye.

Two particular arguments will come up, the first of which is the doctrine of equal misery, thoughtlessly used a year ago as the excuse for reducing public support for British universities by 8.5 per cent over three years. In a memorable display of insouciance, the then Secretary of State for Education and Science told the House of Commons that all public institutions must expect to share the burden of financial sacrifice. He was echoed last Wednesday in the House of Commons by his successor, Sir Keith Joseph. Ironically, if the cost of the enforced redundancies among academics now in prospect falls on the government (and there is no other way in which it can be met), the decisions that have been made will not constitute economies at all. The danger now is that the reduction of support for the universities will be used as an argument for cutting back on the budgets of the research councils. For if the research councils exist in part to support academic research, and if the number of academics is to be reduced, it will be argued that research council budgets can be correspondingly reduced.

That argument is a nonsense which betrays a facile ignorance of the upheaval now under way in British universities. Ostensibly at least, the selective allocation of funds by the University Grants Committee in the summer involved an assessment of individual universities' performance in research. At least in theory, casualties among university departments in the next few years are likely to be heaviest among those with an indifferent record in research. On this assumption, the university departments left substantially intact will be just as much in need of research support in the years ahead as in the past. And if the talents of able academic researchers left stranded in moribund departments are not to be wasted (see Nature 19 November, p.198), the research councils will also need funds to help people migrate to centres where promising research can be carried through. What this implies is that the reduction of public subvention for the universities is entirely irrelevant to the needs of the research councils. And any substantial reduction of the councils' capacity

to support academic research will further endanger the health of the British academic research enterprise, already well below par.

Although the budgets of the research councils have been to some extent protected since stagnation set in a decade ago, the protection is far from complete. The cash limits laid down for the present financial year have not adequately compensated for inflation. Meanwhile, the effectiveness of what the research councils can afford to spend in universities is being steadily undermined. With the collapse of the dual support system, by which universities are supposed to provide basic research facilities out of their own funds, the research councils are willy-nilly having to dip into their own pockets for that purpose. At the same time, they are finding that they can no longer fund all the research proposals of high quality that come their way. The Medical Research Council now says so explicitly. But even the pattern of grant applications in the past few years, when the research councils have congratulated themselves that good proposals have been adequately funded, has probably been misleading. Academic research groups, which are not foolish, have for many years been trimming their ambitions to the public knowledge of what the research councils can and will support. The knowledge that proposals that are too ambitious are unlikely to succeed has already contributed to the despondency in the British research community

Even as things are, the condition of the British academic research enterprise gives the lie to the government's claim that research has hitherto been protected. As the infrastructure is eroded, the research councils are compelled to operate on ever thinner ice. Already they have been forced to postpone plans that would in normal times have been considered vitally important the Science and Engineering Research Council, for example, has just put off a plan to break new ground in the fashionable field of molecular electronics. The danger now is that the collapse of morale among academic researchers will be accentuated by the real decline of resources to support their work. The result, quite quickly, could be catastrophic. Even as things are, the government could find that the lack of enterprise and innovation that has brought Britain to its present economic plight will persist even when the long recession comes to an end. To think of further reductions of the research councils' budgets is to behave as if the chance of economic recovery has now vanished. If that is what the government believes, it should say so.

#### Hunger strike's damage

Andrei Sakharov is on hunger strike. Is he right?

Two years of exile in the city of Gork'ii would be enough to drive anybody to despair. So it is forgivable that even courageous spirits such as Andrei Sakharov and his wife Yelena have embarked on a hunger strike to persuade the Soviet government to agree that their son's fiancée, Liza Alexeyeva, should be allowed to join her future husband in the United States. One snag, as the Sakharovs must know, is that their isolation in a remote part of the Soviet Union to which foreign visitors are not allowed will blunt the effect of their protest. Gork'ii is not, after all, Belfast, where the British government obligingly answered questions from the press about the slow progress towards death of the Irish republicans who fatally starved themselves earlier in the

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year. The Soviet authorities, on the other hand, will have no such obligation.

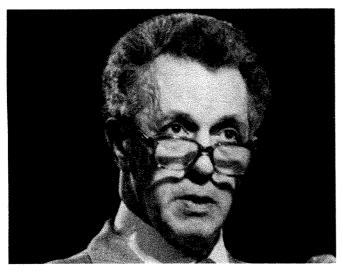
Andrei Sakharov's explanation of his despair is, as always, moving but, on this occasion, strangely unreal. His sense of responsibility derives from having urged his son Alyosha to emigrate when a brief opportunity appeared in 1978, leaving his fiancée behind. (They were later married by proxy in Montana.) But the Soviet authorities are habitually so slow to grant exit visas to citizens wishing to join foreign spouses as to support the belief that they are not willing to do so. The Sakharovs know this, and must also suspect that the sacrifice on which they have embarked may prove fruitless: if they should die, Liza Alexeyeva will be even less well-placed than at present. The Sakharovs' courage is admirable and beyond dispute. Is it possible that on this occasion their isolation has led them to misjudge the future?

Andrei Sakharov's explanation lends some support to that unhappy guess. He complains that appeals for help with his immediate problem addressed to four members of the Soviet Academy have come to nothing, and that one of those asked — Academician Ya. Z'eldovich — had flatly refused to help on the grounds of the "shakiness of his own position". Sakharov goes on to accuse Z'eldovich of abandoning responsibility. It is, however, well-known in the West that Z'eldovich, a distinguished theoretical physicist turned cosmologist, is among that large company of Soviet scientists unable to accept invitations to conferences abroad. Sakharov's own protest against illiberality has hitherto been entirely admirable. In the loneliness of his exile, it is natural that he should be looking to others for help. What they do, and how they do it, is nevertheless for them to decide. That the Sakharovs themselves have fallen back on the hunger strike is especially distressing, not merely because of the risks involved but because the hunger strike is essentially an irrational form of protest. Governments may yield on particular points, but are unlikely to recast their policies as a result. More often, they dig in their heels, declining to give in to emotional blackmail.

#### Academic cat and mouse

Can Sir Keith Joseph, the least successful minister in the British Government, be trusted with the universities?

The proposition that higher education is too important to be left to educationists would be widely accepted. But can the future of British higher education safely be entrusted to Sir Keith



Joseph, the present Secretary of State for Education and Science? In a debate last week in the House of Commons, Sir Keith uttered this perplexing statement:

Those who maintain that it would be educationally less harmful but still productive of the same savings to go slower have not made out their case. I concede that logically such a case could be made, but I do not believe that it has been made.

If one is made I shall consider it.

If words, even as interpreted by *Hansard* shorthand writers, mean anything, he was saying that British universities have not yet found the forms of words with which to defend themselves against the folly of his government's decision that the university budget should be reduced by 15 per cent in three years At the same time, however, Sir Keith implied that if only academics were as smart as he is, they would get their act together and persuade him, as he secretly knows they could, that their case for a less draconian contraction makes economic as well as academic sense. And secure as he is in the kmowledge that no British academic will pass that stringent test, he will let the system founder.

Sir Keith has a curious reputation, more that of an intellectual than of a politician. His spell as Secretary of State for Industry is memorable chiefly for his advocacy of the importance of market forces and for his contradictory willingness to make out a cheque for whichever nationalized industry was then in trouble. Last week, he rightly challenged his critics to explain how an openended commitment by British governments to meet the cost of educating all qualified entrants could be reconciled with the limited capacity of taxpayers to meet the cost. The answer (which follows) is not arithmetical.

Sir Keith was right last week implicitly to say that no British government could permanently agree that all "qualified" students should be educated free, in the disciplines of their choice, at the taxpayers' expense. He should also have done the decent thing and said that Robbins, as a principle, is dead. What bothers the government about the open-endedness of its inherited commitment, however, is not so much the cost of operating the universities as that of paying maintenance grants to students. Sir Keith's officials are already negotiating with the National Union of Students on the students' demand that their maintenance grants should be increased by nearly 18 per cent. The officials will have at least one hand tied behind their backs by the legislation that compels each local authority to pay maintenance grants to students whenever they are awarded places at institutions of higher education. The profligacy of this legislation is, outside the United Kingdom, beyond belief. Not merely does it constitute Sir Keith's chief problem but it is the albatross hung around the neck of the university system. Replacing the present system of mandatory student grants, limitless in cost, with a system of scholarships awarded to deserving people would help not merely to solve the universities' problems but would get Sir Keith Joseph off the hook on which he has quickly (but characteristically) impaled himself. Unhappily, neither the universities nor the minister supposedly responsible for their welfare have the stomach for such a fight.

#### Monument for a giant

Sir Hans Krebs died this weekend in Oxford.

It is quite true that in the 1930s, *Nature* declined to publish Sir Hans Krebs's article on what most people have since called the Krebs cycle. The explanation given was that the article should be placed in a more specialized journal. Krebs himself cherished the rejection slip but modestly and confusingly always referred to the biochemical cycle he first described by its original name, the "citric acid" cycle (see *Nature* 291, 381; 1981).

Krebs, a refugee from Nazi Germany, carried with him Warburg's way of regarding the biochemical problems of metabolism and, perhaps more important, Warburg's techniques. But only Krebs's flair can account for his unmasking of the intricacies of the most rudimentary of the metabolic processes in animal tissues — the oxidation of sugars to form ATP — with the techniques of half a century ago. Inevitably, that single piece of work will be his chief monument. Another is in danger of being overlooked. Krebs, naturally a magnet for students, modestly bent his energies to teach them all he knew. At one stage, more than a dozen British chairs of biochemistry were occupied by his students. He was a giant eager that his shoulders should be used by younger people.

## MIT agrees to accept Whitehead grant

## Faculty votes yes, but some strings remain

Washington

Despite strong objections from several members of its science departments, the full faculty of Massachusetts Institute of Technology (MIT) voted by an eight-to-one margin last week to approve MIT's acceptance of an offer from multi-millionaire Edwin (Jack) Whitehead to set up an independent biomedical research centre associated with the institute.

The faculty's approval gives a green light to the MIT Corporation and the institute's president, Dr Paul E. Gray, to accept Mr Whitehead's offer of an initial \$20 million for the capital costs involved in building the research centre, and an eventual endowment of \$100 million to cover operating costs. The centre's main goal will be to pursue research into the applications of molecular genetics to developmental biology.

The money will allow the centre, which will be headed by Nobel laureate and MIT biology professor David Baltimore, to recruit at least 20 full-time professors. All will be members of MIT's biology department, since although funding for the centre will be controlled by members of Mr Whitehead's immediate family and other trustees nominated by him, the centre will for academic purposes be treated as an integral part of MIT.

When details of Mr Whitehead's original offer became known early in the summer, several faculty members complained that research staff at the centre would be subject to a "dual allegiance" that could lead to tensions between them and other MIT academics (Nature 1 October, p.329). Concern was also expressed at the original proposal made by Mr Whitehead - cofounder and president of the laboratory instrument company Technicon, which was bought by Revlon in August 1980 in a deal that involved the transfer of \$300 million in Revlon stock to him - that the research centre should own all the patents originating from its work and benefit directly from their licensing.

In the intervening period, the terms of the proposed arrangement have been modified to give MIT more direct control of the centre. Thus all new faculty members appointed to the centre will be required to adhere to existing MIT rules on salaries and benefits, and fulfil the conventional teaching and committee responsibilities of other MIT academics.

Mr Whitehead has insisted, however, that the funds for the centre, to be known

as the Whitehead Institute for Biomedical Research, remain under the control of its finance committee and that his three children should remain a majority on the committee. The conventional arrangement is for a benefactor to provide funds directly to a university, but he says that if he had wanted to give the money to MIT and let the institute decide how to spend it, he would have done so.

Not all the critics have been satisfied with the concessions that Mr Whitehead has agreed to. In a letter circulated before last week's meeting, 33 faculty members expressed their continuing "deep concern" about the implications of the proposed arrangements. The letter, drawn up by physics professor Anthony French and biology professor John Buchana complained that the centre's staff would be selected primarily for their potential contribution to its research activities rather than in order to meet MIT's educational needs; that the creation of the centre would lead to an imbalance in the biology department, since it would provide onethird of the full professors; and that there would be a lack of symmetry between the voting power of the centre's scientists over the work of the rest of the biology department and vice versa.

MIT officials, however, are keen to accept Mr Whitehead's offer, which they see as helping to keep MIT in the forefront of biological research and its potential

commercial application. In a letter circulated to all faculty members before last week's meeting, Dr Gray and MIT provost Francis Low said they shared the general concern about the potential problems that could arise as a result of the dual loyalty of faculty members. But they added: "Although this risk cannot be eliminated, in our view the worries are based on worst-case scenarios that are not likely to materialize".

After a sometimes stormy debate, in which Dr Gray said he did not know the detailed reasons why a similar offer from Mr Whitehead had been turned down by faculty members of Duke University in North Carolina in 1974, a motion to support the creation of the research centre was approved on a straw vote by a majority estimated as eight-to-one. At the same time, the motion said that members of the faculty "acknowledge the existence of legitimate, deep concern over the risks inherent in the venture, and hope that efforts to minimize these risks will continue".

Mr Whitehead's offer will now be formally considered by the members of the corporation, the controlling body of MIT, when they meet on 4 December, and is expected to be accepted. Dr Baltimore expects the new institute to get under way as soon as the affiliation had been formally approved by the corporation.

.. David Dickson

#### Mitterrand embraces information policy

M. François Mitterrand, President of France, is wasting no time responding to the "information shock" — the complex of issues surrounding microelectronics and unemployment.

In June, Mitterrand asked the publisher Jean-Jacques Servan-Schreiber (who had written a book on the subject) to gather a few experts to study the question. Servan-Schreiber's response came last week in a report which proposed the setting up of a world centre in Paris. This would gather together international experts in informatics, and have a budget of around £10 million a year (£4 million of which would pay the 60 staff). Within the week, Mitterrand had agreed and ordered that the details should be worked out within a fortnight. So the World Centre on Informatics should be in action in 1982.

What it will do, however, is not clear. Servan-Schreiber's vision is of a transformation of the world mediated by the development of the personal computer (something cheaper and more accessible than the present office and household machines). Such computers could make work more varied and creative, and provide everyone—including the Third World—with almost unlimited access to information. Development is a matter of

education, he says, and the personal computer can facilitate it. The world centre, therefore, should observe and assist this transformation.

Mitterrand, however, is aware that in industry the application of microelectronics might cause unemployment, and that the computer can interfere with liberty and privacy. At the same time, he considers that the economic development of France, and of other Western nations may depend on the rapid implementation of microelectronics and informatics. Elected to conquer unemployment, and determined to increase his country's economic strength, he needs an answer to this dilemma and hopes that the world centre, as a kind of full-time standing committee of academics, will provide it.

Although the precise form of the centre has yet to be determined, the staff is certain to be international. So far, ten experts have expressed interest in joining — seven from America, two from Scandinavia and one from China

If the centre develops as Mitterrand wishes, it will:

- Monitor the applications and effects of microelectronics worldwide.
- Forecast the possible role of France in these developments.

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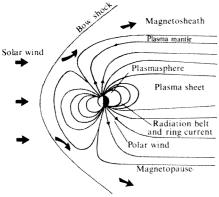
- Study how to educate users and potential users of microelectronics, particularly in relation to maintaining employment.
- Help develop new programs and languages appropriate to a personal computer (Servan-Schreiber version).
- Propose the means of transferring the technology to the Third World.

Robert Walgate

#### Magnetospheric experiment

#### UK joins in

The Science and Engineering Research Council has decided to fund participation by the United Kingdom, to the tune of £2 million, in the Active Magnetospheric Particle Tracer Explorers project (AMPTE). The council's Rutherford Appleton Laboratory, in partnership with the Mullard Space Science Laboratory of University College London and other university groups, will be developing the satellite as a separate component of the Ion Release Module satellite being developed by the Max Planck Institutes of



Astrophysics and Aeronomy in West Germany. The two satellites, together with the Charge Composition Explorer satellite being developed in the United States, will all be launched in mid-1984 on a Thor Delta rocket, hastily acquired following space shuttle delays.

The magnetosphere is the region where the magnetic field of the Earth interacts with the charged particles and magnetic fields of the solar wind, the continuous flow of plasma from the Sun. The aims of the experiment are to release lithium and barium ions into the Earth's magnetosphere and, by monitoring their subsequent behaviour, to investigate several fundamental aspects of the magnetosphere as well as to simulate conditions near other planets. The Ion Release Module will be placed in a highly eccentric orbit, so that ions may be released both inside and outside the magnetosphere.

One of the problems to be tackled by the Charge Composition Explorer is to determine whether or not the magnetosphere is "open" — that is, whether the Earth's magnetic field lines can connect with those of the interplanetary medium so that solar plasma can enter the Earth's field

from downwind. To this end, ions will be released just upstream of the bow-shock. The Charge Composition Explorer, in an equatorial orbit, will detect those ions only if the magnetosphere is "open".

The UK satellite will be placed in an orbit with that of the release module, maintaining a separation of 100 km or so. The particle and field detectors on board will monitor the behaviour of the ejected ions immediately after release. Lithium ions, being light, should pick up a large component of the velocity of the solar wind unless disturbed by plasma instabilities.

The release of the barium ions should produce rather more spectacular results. Because they are more massive than most components of the solar wind, the surrounding plasma will be impeded and a glowing comet-like structure will be produced. If the barium ions are released within the high-velocity plasma of the magnetosheath near dawn or dusk, the cloud will be visible from the ground, and will simulate conditions to be found in comets and in the magnetosphere of Venus. When released "downwind" in the magnetotail, where plasma flows more slowly, conditions will be more similar to those near Io and Titan within the magnetospheres of their respective planets Jupiter and Saturn. **Philip Campbell** 

#### NASA budget

#### Small is necessary

Washington

As the National Aeronautics and Space Administration (NASA) locks horns with the Reagan Administration over just how large a budget cut it must face next year, US space scientists are trying to devise a strategy for exploring the Solar System within the tight budget constraints likely to dominate for the next few years.

For the fiscal year beginning 1 October 1982, the Office of Management and Budget (OMB) has told NASA to prepare for cuts of about \$1,000 million in a budget previously projected at about \$7,000 million. Agency officials are strongly resisting this request, arguing that such a cut would severely damage all NASA's programmes; they have already gone over OMB's head to the White House in an attempt to get the proposed cuts reduced before the budget is formally submitted to Congress in January.

However, the prospects for NASA in general — and space science in particular — are likely to be bleak for the next few years. In this context, the agency has set up a Solar System Exploration Committee to take a close look at the type of projects it should be planning for the future. "If something disastrous were to occur to NASA's budget next year, it would be even more important to plan how we should recover", Dr Noel Hinners, the committee's chairman and director of the National Air and Space Museum in

Washington said last week.

The main aim of the committee is to increase the efficiency of planetary and other Solar System missions while reducing the costs, in particular by breaking down the scientific goals of the missions into "smaller bites" than those represented by, for example, Voyager.

One way of achieving this goal would be to build directly on the experience of previous missions. For example, the Ames Research Laboratory is looking at ways of developing Pioneer-type orbiters to some of the inner planets, using derivatives of terrestrial orbiters to study the geological characteristics of the Moon or Mars.

Pioneer-type spacecraft would only be able to achieve relatively limited scientific objectives, and would produce a relatively low data rate. But a mission could be launched for between \$100 and \$150 million, the same order of magnitude as the Explorer satellites currently being successfully used by NASA.

As well as looking at ways of using smaller spacecraft, the committee is investigating how to save money by increasing operations efficiency, for example by reducing the length of trips, compressing the scientific data to be transmitted back to Earth or the sharing of individual pieces of equipment between different research groups.

Four subcommittees will meet next month at the Jet Propulsion Laboratory to work out the type of programme that could be developed on the basis of such components. The subcommittees expect to report to NASA with specific mission proposals by next summer.

Dr Hinners said last week that he had discussed this new approach in some detail with officials at OMB, who expressed general approval but raised two possible objections. First that a list of the various missions which could be included in an overall envelope of between \$300 and \$400 million a year — only slightly above the current level — looked like an ambitious shopping list; and second, that breaking down planetary missions into smaller projects might lack the public appeal of Apollo or Voyager.

To the first objection, NASA replies that it is the size of the effort and the balance between costs and return that should be measured rather than the number of space vehicles flown. The second objection raises a broader question faced by NASA: can the apparently-increasing enthusiasm among the public for space exploration, particularly in the light of the excitement generated by photographs sent back by the Voyager spacecraft, be interpreted as a mandate to increase spending on space science?

Those who do believe that the public will be eager to support ambitious plans for space exploration are still pushing hard for what they consider should be the next major mission, a manned sample-return space vehicle to Mars. NASA administrator James Beggs told the advisory board last week that he shared the enthusiasm for such a project, although arguing that it should be planned in conjunction with what is likely to be NASA's next major project, a permanent orbiting space station which could be used as a base for manned planetary missions.

Members of the advisory board wondered whether there might be a danger that if NASA did embark on such a project it might further squeeze the space science budget, as the Apollo Moon landings and the space shuttle appeared to have done, but Dr Beggs tried to calm such fears. Admitting that the near-term looked relatively bleak, with no new planetary starts likely to be approved for 1983 or 1984, he replied that in the past the space science budget had done best precisely at those times when NASA had been able to generate political support for its major undertakings. **David Dickson** 

Diablo Canyon reactor

#### Licence revoked

Washington

The US nuclear industry has come in for an unexpected roasting from the new chairman of the Nuclear Regulatory Commission (NRC), Dr Nunzio Palladino. His criticism of its quality control was made before a congressional committee on the same day that the NRC voted to revoke a licence issued only two months ago to permit start-up tests at the Diablo Canyon nuclear reactor in California, on the basis of a series of engineering mistakes made when the plant was strengthened to resist earthquakes in the mid-1970s.

Six weeks of frequently-violent demonstrations by anti-nuclear protesters in September and October failed to stop the plant's owners, Pacific Gas and Electric Company, from proceeding with plans to start to load the reactor with uranium. The protesters claim that the plant is inherently unsafe because it has been built less than three miles from the Hosgri Fault, a branch of the San Andreas Fault system.

As the demonstrations were coming to an end, however, an engineer with the company discovered that a blueprint had been misread when the plant's support structure was being strengthened to compensate for the possibility of earthquake damage, causing the loads on various components to be miscalculated. Further errors found later included the misapplication of stress level numbers along the Hosgri Fault and the use of incorrect data in calculating the ability of pipes to withstand an earthquake.

Besides causing considerable embarrassment to the Pacific Gas and Electric Company, this discovery led to immediate pressure on NRC from Congress. The Reagan Administration has promised to speed up the licensing of new plants; but this strategy requires that public confidence should be maintained that safety is not being sacrificed in the process.

Appearing before the House Interior subcommittee, Dr Palladino, former dean of engineering at Pennsylvania State University, said that "after reviewing both industry and NRC past performance in quality assurance, I readily acknowledge that neither have been as effective as they should have been in view of the relatively large number of construction-related deficiencies that have come to light".

An order approved by the five-man commission, which voted 4-1 to suspend the low-power operating licence and unanimously for an independent audit of the quality control safeguards used by Pacific Gas and Electric Company, said that the suspension was necessitated by the seriousness of the errors in the initial review of safety modifications.

Following NRC's decision, the Pacific Gas and Electric Company issued a statement saying that it was "disappointed", since "nothing has been discovered to date that would indicate that the plant is not safe". The company claims that the plant had many redundant safety systems compensate for any threat from the Hosgri Fault.

David Dickson

#### More bickering about solar satellites

Washington

The National Aeronautics and Space Administration (NASA) seems unlikely to grant the request from the European Space Agency (ESA) to bring forward the launch of the International Solar Polar Mission, now due in 1986.

ESA had made this request following NASA's decision to stop work on the vehicle which it was to have provided for a dual-spacecraft mission, with the original intention that the two would pass simultaneously in opposing orbits over the poles of the Sun. European scientists had hoped that NASA, which will still launch the ESA space vehicle from the space shuttle, would be able to arrange an earlier flight to help compensate for the disruption and the loss of experiments which the cancellation has caused.

Last week, however, NASA officials said that the growing concern over whether the shuttle will be able to maintain an already overcrowded launch schedule means that a 1984 launch is virtually out of the question.

There is a slight possibility of a launch in 1985. However, since there is only a relatively short launch window in that year, and since the same window is required by the Galileo probe and orbiter scheduled to start its journey to Jupiter at the same time, the chances of arranging both launches within the same period seem slim.

ESA officials are still angry at the way in which NASA cancelled its proposed spacecraft; a resolution adopted by ESA's space programme committee last month suggested that ways should be explored of seeking some type of compensation from the US agency and ESA's secretary general Dr Quistgaard suggested the early launch date as one form of recompense.

US officials admit that it is the first

time NASA has had to go back on a previous agreement, but claim that the "memorandum of understanding" signed between the two organizations makes it clear that the solar mission agreement was subject to the availability of funding, and that NASA could not legally commit itself to more than one year's advance funding.

In addition, Dr Hans Mark, deputy administrator of NASA, told a recent meeting at the National Academy of Sciences that although the decision to cancel NASA's involvement in the solar mission was regrettable, there had been several occasions in the past when European governments had broken commitments, for example some of those made through the North Atlantic Treaty Organization.

On the first point, ESA claims that the memorandum of understanding had been agreed on the basis that normal funding procedures would be pursued by both sides — and that NASA is at fault not for having failed to provide the funding promised, but for having decided unilaterally to omit the project from its request to the Office of Management and Budget for the 1983 budget. On the point that some European organizations have broken commitments in the past, ESA argues that it is not fair to penalize ESA for failures of individual European states.

Meanwhile NASA scientists are discussing the possibility of launching an Explorer satellite into an ecliptic orbit around the Sun to coincide with the European spacecraft's encounter. This satellite, which would be launched in 1985, would provide baseline measurements of the solar wind — and that might go some way to making up for the deficiencies resulting from the decision not to send a full US spacecraft.

David Dickson

#### Uranium enrichment

#### **US-India** stand-off

New Delhi

India is trying to become self-sufficient in the production of fuel for its nuclear power plants following its bitter experience with the United States over the supply of low enriched uranium for the Tarapur plant in Maharashtra state.

The latest round of discussions between Indian and US officials has failed to break the impasse over the clearance of at least pending applications with the United

States for the supply of enriched uranium. The United States maintains that India should sign the Nuclear Non-Proliferation Treaty and open all its nuclear installations — indigenous as well as foreign-aided — for international inspection as required by the US Nuclear Non-Proliferation Act of 1978. The supposed fear is that a uranium reprocessing facility in India might be used to extract plutonium for atomic weapons.

India rejects this contention, however, arguing that the 1978 US legislation should not be applied retrospectively and unilaterally to a bilateral agreement entered into in 1963. India has said time and time again that its nuclear technology would be used for peaceful purposes only. India holds the Nuclear Non-Proliferation Treaty to be discriminatory, saying it includes only civilian establishments and specifically excludes military establishments of the nuclear weapon states which prescribe non-proliferation for others and not for themselves.

The issue is now a matter of principle — especially as India is now almost self-reliant for nuclear fuel production.

Indian nuclear scientists have developed mixed oxide fuel of uranium and plutonium which can work as alternative fuel in place of the enriched uranium supplied by the United States for the Tarapur plant. The only other operational nuclear power plant at Kota in Rajasthan utilizes indigenous natural uranium. The nuclear plants being built at Narora and Kalpakkam will also be pressurized heavy water reactors using indigenous uranium.

Sunil Saraf

#### US nuclear technology

#### Exports raise fears

Washington

Fears are mounting in Washington that the Administration's efforts to increase nuclear technology exports could be encouraging the proliferation of nuclear weapons. Last Thursday, members of Congress questioned the Administration closely about its agreement with Australia which, for the first time, would mean the United States sharing its knowledge of centrifuge technology for enriching uranium.

The criticism came only a few days after a new storm had broken over the ability of the International Atomic Energy Agency (IAEA) in Vienna to provide satisfactory safeguards against the diversion of nuclear materials from civilian to military use.

The decision to share enrichment technology with Australia is part of an effort to encourage US companies to participate in a joint venture with the Australian government to construct enrichment facilities for its nuclear industry. It was contained in a memorandum signed on 12 November by President Reagan which also instructed the Department of Energy to look at ways of

#### British academics at the barricades

Genteel academic militancy reached boiling point last week, with a mass lobby of the British Parliament by some 10,000 university teachers protesting not merely at the British government's decision that the university budget should be cut but at the uncertainty that remains about the arrangements that may (or may not) be made to deal with redundancies among academics. Some of the participants (see picture) wore fancy dress.

The lobby (on Wednesday, 18 November) coincided with a debate in the House of Commons on the planned reduction of the public subvention for universities, called by the Labour



opposition. One government speaker complained that it would have been more convenient if the debate had been arranged for the following day, so that those inclined to do so would have had a chance to listen to what the lobbyists were saying.

Both occasions followed by a lunch-time break the first appearance of Sir Keith Joseph, the new (since last month) Secretary of State for Education and Science, before the Select Committee on Education, at which he and his retinue of civil servants were unable to put into words a definition of the "Robbins principle", the doctrine that qualified candidates for university entry should be catered for. At the beginning of last week, the UK Committee of Vice-Chancellors also (unusually) made public its own account of an unsatisfactory meeting with the minister and a waspish letter it had written to him afterwards.

The debate in the House of Commons has confused and not clarified the immediate financial prospects of British universities. Sir Keith Joseph and his minister with special responsibility for higher education, Mr William Waldegrave, declined to answer the apparently simple question whether the government would pay the cost to universities of breaking contracts with tenured academics. Each of them said, however, that the British government would be prepared to "listen to" arguments that it would save money by extending the period over which the universities were now required to contract.

The Committee of Vice-Chancellors is now drafting such a document.

transferring the federal uranium enrichment programme into private hands.

During a hearing of the Senate Foreign Relations Committee's subcommittee on energy and nuclear non-proliferation, several members questioned Administration officials closely on this decision. Centrifuge technology has previously been subject to strong government restrictions, on the grounds that it could provide a relatively inexpensive way of producing weaponsgrade nuclear fuel.

However, the Administration continues to insist that, although a hard line will be taken with any country that diverts civilian technology to military use, in general IAEA provides the best way of minimizing the risks of proliferation through its safeguards and regular inspections.

This argument suffered a setback earlier this year when an ex-IAEA inspector, Mr Roger Richter, told the same Senate committee that IAEA had failed to detect efforts by the Iraqi government to work clandestinely on nuclear weapons, and that present IAEA safeguards were "totally incapable of detecting the production of plutonium in large-size material test reactors".

At the time, IAEA officials fiercely contested Mr Richter's conclusions, claiming that he had not been aware of all the relevant facts. However, it now looks as if they will have to go through the same process in defending themselves against criticisms made by another ex-inspector, Mr Emanuel R. Morgan, in a report commissioned for the Nuclear Regulatory Commission by commissioner Mr Victor Gilinsky.

The report — not officially released but leaked to the *New York Times* — echoes Mr Richter's conclusion that IAEA is incapable of detecting the diversion of a significant quantity of nuclear fuel "in any state with a moderate to large nuclear energy establishment".

This memorandum, likely to be discussed shortly in a congressional hearing, is the first critical assessment of IAEA's safeguards to have been prepared for a federal agency. The State Department issued a statement saying that although it accepted that the safeguards system was not perfect, there was "simply no alternative to an international safeguards regime".

David Dickson

#### Product development

#### British battle on

Brussels

The British government is fighting hard to exclude development risks from the EEC's draft directive on product liability now being debated in Coreper — the Committee of Permanent Representatives to the European Community. This has caused great consternation among the European consumer lobby group, Beuc. The consumer group fears that the exclusion of development risks would undermine the whole system of direct liability, by reversing the burden of proof and placing it on the consumer.

The alternative to development risks, the state-of-the-art defence, means that if the manufacturer can prove that he took all reasonable care in the light of the state of scientific and technical development to ensure that his product was safe, he avoids paying compensation. But who then compensates the consumer for his loss if a product, such as thalidomide, turns out to be dangerous? In the United Kingdom the Pearson Royal Commission and the Law Society, concluded that the manufacturer should bear direct liability for development risks. The Council of Europe has come to the same conclusion and France, Belgium and Luxembourg have signed a convention to that effect.

Belgium takes over the presidency of the Council of Ministers from Britain next year and the United Kingdom is anxious to have this part of the directive dealt with by then. The British argue, following pressure from the pharmaceutical industry, that development risks inhibit innovation and competitivity. In the last debate on this subject in the House of Commons, Sally Oppenheimer, the Minister for Consumer Affairs, argued that the EEC proposals would involve a major change for British manufacturers.

The experience in other EEC countries shows that the change is not as painful as alleged. For instance, the German law on pharmaceutical products provides for direct liability and manufacturers cover themselves by taking out an insurance amounting to 2 per cent of turnover.

The British are, however, confident that a compromise can be reached. This could involve excluding development risks or limiting them to pharmaceutical products.

Jasper Becker

US solar energy

#### Golden housing

Washington

Denying charges that the Reagan Administration is ideologically biased against solar energy in favour of nuclear, US Energy Secretary Mr James Edwards last week announced the go-ahead for the Solar Energy Research Institute (SERI) in Golden, Colorado, to start building a permanent research and test facility.

The Administration's decision is its first bit of good news for supporters of solar energy, whose budget within the Department of Energy has dropped from the \$707 million proposed by President Carter to less than half that in the current year.

Much of this reduction in funds had been felt at SERI. This institute, which is run for the Department of Energy by the Midwest Research Institute, has already had its budget cut from \$120 million to \$50 million, and staff reduced from \$60 to 650.

Since its creation in 1977, SERI has occupied temporary premises in the town of Golden, just outside Denver, Colorado. But it has also been planning a combined laboratory and showcase, to be located on a nearby mesa on land donated by the state.

Initial proposals for a solar research and demonstration centre costing \$124 million were reduced by the Carter Administration, which then requested \$24 million in the 1981 budget for the first stage of construction. One of the first acts of the Reagan Administration was to withhold all construction money on the grounds that it was re-evaluating its whole approach towards funding solar energy research.

At one point, it was rumoured that the Office of Management and Budget was contemplating eliminating all funding for such research from the Department of Energy's budget. Publicly, however, the Administration has stated that although it is eliminating demonstration projects which it feels ought to be supported by the private sector — if at all — it accepts the need for federal support of long-range, high risk research.

The permission from the Department of Energy for construction of SERI's laboratory is being used by the Administration to symbolize a commitment to solar power. But Mr Edwards said that the \$9.5 million which he was making available for the initial construction phase — money from appropriations previously authorized by Congress — did not necessarily mean extra funds for energy research.

But the decision to fund SERI's laboratory does not necessarily mean that federal support for solar energy research will be generous. The rumour in Washington is that the Department of Energy's budget request for 1983 contains a meagre \$93 million for all solar energy work, a contraction to the level of the early 1970s.

David Dickson

#### Californian Medflies

#### **Innocent victims**

Palo Alto, California

If aerial spraying with the insecticide malathion is used to counter the threat of Mediterranean fruit fly in California this winter, an unexpected casualty might be one of the oldest continuously studied insect populations in the world.

Jasper Ridge is a 1,200-acre natural wilderness to the west of the Stanford University campus in California, between suburban San Jose and the city of San Francisco. More than 120 dissertations and papers have been written about the area since 1897 when studies began in earnest. So far, Jasper Ridge has been a virtually unsprayed island, surrounded by a 600-foot buffer zone. But in July spraying began in areas next to the reserve.

Most immediately at risk, of course, are the insects themselves, notably the checkerspot butterfly *Euphydryas editha*, which has been the subject of a continuous population study since 1960, under Professor Paul Ehrlich.



Paul Ehrlich may search in vain next year

Although Jasper Ridge has not been sprayed directly, minute droplets of malathion bait (20-40 µm) have been found on plant leaves within the reserve. and these droplets must have drifted into the area from nearby spraying operations. During the summer, the drifting droplets did not appear to affect the checkerspot butterflies, which lay dormant under ground. But in the rainy season the checkerspots emerge to feed, and any direct spraying during the winter would almost certainly mean their complete disappearence from Jasper Ridge. Aerial spraying has been curtailed for the winter months, but will be stepped up again next spring (see Nature 12 November, p. 103).

Direct spraying would almost certainly be carried out, however, if two adult Medflies, or a pupa, were found within the Jasper Ridge reserve. The ecological balance of the reserve would then be irretrievably disturbed by an uncontrollable variable. Donald Kennedy, a biologist and Stanford University president, compares the possible loss of Jasper Ridge with the loss of an irreplaceable library. Charlotte K. Bevers

#### CORRESPONDENCE

#### Alchemy in cancer

Sir — Your editorial (*Nature* 5 November, p.1) describing the attitude of the sponsors of cancer research in the United States towards those who perform the research was most timely. The relationship between sponsor and sponsored in research generally is presumably similar except that in cancer research the goal is more visible and unease at the failure to achieve it more acute.

Without wishing to push the analogy too far, there is an apparent similarity in the relationship between workers in cancer research and their sponsors on the one hand, and on the other, the alchemists pursuing the philosopher's stone at the behest of their princely masters. It is a sobering thought that many alchemists lost their heads for failing in their sphere of endeavour. Fortunately, nowadays, it is only our jobs that are at risk.

NEVILLE WILLMOTT

Department of Oncology, University of Glasgow, UK

#### Creationism

SIR — Our letter "American Creation" (Nature 2 July, p.95) appears to have resulted in two misunderstandings. The first, implied by the misleading title, is that it was primarily a critique of American Creationism. It clearly was not. The second, that we argue for a "God of gaps" by resorting to the supernatural to fill the gaps in current scientific knowledge, requires more serious consideration, since we agree with Sidney Fox (Nature 6 August, p.490) that such a view is a "copout" scientifically and theologically.

In common with most creationists, we acknowledge that faith is based on revelation and on personal knowledge of the Creator The only empirical evidence to support the assertion that all nature, however adequately or inadequately explained, is God's handiwork, is the fact that all things exist, and show evidence of design and purpose which reflect the revealed nature of the Creator. Given belief in such a Creator we expect to find evidence consistent with his revelation of himself. No doubt an atheist will likewise expect to find evidence consistent with his philosophical position and is likely to leave discordant data to one side in the belief that future study will show it to be either erroneous or, given further data, assimilable to his present beliefs. In that sense it is indisputable that since scientific knowledge is incomplete. gaps exist in any account of the natural world.

Furthermore we would argue that the primary purpose of man's quest to understand origins is philosophical not scientific, and that in a society that will not tolerate a personal infinite God, the purpose of evolutionary theory is often the justification of atheism. In this context it is critical that scientific evidence be analysed within a completely objective framework, before presuppositions are imposed upon it.

Our position is that we believe that existing evidence does not disprove the existence of a Creator and further that the most probable explanation of his activity as Creator is one which does not accord with current evolutionary theory. In examining the factual evidence available in biology we consider that the correct objective approach to biological

systematics is to employ cladistics, a methodology which might usefully be applied also to protein and nucleic acid sequences. The interpretation of such an analysis is subjective, resting on philosophical presuppositions. So any evolutionary relationships that exist are properly deduced from cladistics.

So far as the origin of life is concerned we suggest that the most likely interpretation of scripture is that life did not evolve from a prebiotic soup. Thus we are not surprised that there are difficulties in proposing plausible chemical mechanisms to support the spontaneous generation position. For example, to take Fox's experimental model, we agree that while plausibility can be argued indefinitely, nonetheless the only basis on which the plausibility of such a model may be assessed, however subjectively, is by an examination of the experimental data. Although we do not think it appropriate here to enter into detailed technical comments, we find Fox's data (Biosystems 12, 55) singularly unconvincing.

> C.H. Darnbrough J. P. Goddard W. S. Stevely

University of Glasgow, UK

#### Malaria debated

SIR — I have read the paper by Chapin and Wasserstrom (*Nature* 17 September, p.181) with interest. I am disappointed with the presentation and discussion of the important subject of malaria resurgence and its relationship to agricultural production.

The authors give a garbled account of the very concept of malaria eradication and especially of the causes of the relative failure of this great endeavour. They imply that the main obstacle to the early achievement of the planned goal was resistance of Anopheles to insecticides. This is not so, even though the latter phenomenon played a significant part in technical problems that the World Health Organization (WHO) was facing during the late 1960s. The most comprehensive analysis of the multiple causes of the disappointing progress of malaria eradication in some parts of the tropical world was presented by WHO in 19691. It pointed out that administrative, social, economic and financial factors were largely responsible for the resurgence of malaria at the end of that decade, especially in

There is nothing "ironical" in the fact that agriculture in India and elsewhere expanded in regions where malaria incidence decreased spectacularly. It is precisely because of the improved health conditions, due to the use of residual insecticides against *Anopheles* vectors, that efficient agriculture became possible not only by rich landlords but also by small farmers. The astounding graph showing the positive association between the use of DDT in India and the striking increase of malaria in 1969–77 cannot be accepted in support of the authors' thesis of a causal relationship between the two factors.

An epidemiological analysis of the resurgence of malaria in India, such as the one carried out by Akhtar and Learmonth<sup>2</sup>, showed that the increase in the incidence of malaria in India from 350,000 cases in 1969 to nearly 2 million in 1973 and then to 5 million

in 1975 was due to other conditions with an adverse affect on the standard of anti-malaria operations in India and in other countries of the subcontinent. The military conflict with Pakistan, the sharp fall in the flow of American aid and the temporary food shortages due to bad harvests took place during that period. There were delays in the allocation of foreign exchange for insecticides and drugs and there was some loss of urgency of the malaria control campaign because of its good results and the emphasis on family planning.

The successful 1965-69 phase of malaria eradication in India left uncompleted four large areas from which the consequent resurgence spread. These were the Rann of Kutch in western India, Madhya Pradesh hill forests. Orissa hill forest tracts and the forested areas of Assam. Akhtar and Learmonth<sup>2</sup> indicated that densely populated areas, with extensive irrigation and high agricultural production, have shown less malaria than other areas during the period 1970-75. On the other hand, in spite of the continuous use of DDT for agricultural needs, the amount of malaria in India decreased sharply between 1977 and 1980, thanks to better implementation of control programmes.

A series of valuable studies by Indian experts<sup>3,4</sup> and an Audit Report of the Agency for International Development<sup>5</sup> attach much less importance to the problem of insecticide resistance and could be quoted as an argument against the thesis so eagerly adopted by Chapin and Wasserstrom.

The adverse effects of excessive use of residual insecticides in agriculture have been known and stressed by WHO for well over a decade. It is quite untrue that WHO did not urge all countries in the developing world to decrease as much as possible (without endangering their food and health programmes) the use of residual insecticides and to introduce alternative methods of pest control. The emphasis on this policy was clearly stated in the 16th Report of the WHO Expert Committee on Malaria<sup>6</sup> and was repeated and emphasized in the 22nd Report of the WHO Expert Committee on Insecticides7 and in every other relevant WHO report.

The serious imputation that the Food and Agricultural Organization (FAO) policy is being influenced by large insecticide-producing companies will certainly be answered by those accused of irresponsibility or other more sinister intentions. The authors use with evident relish the impressive term "integrated control" although it is doubtful that they fully understand its practical implications. Integrated pest management means a combination of chemical, biological and environmental methods. The complexity of implementation of these methods is often difficult to fathom by non-specialists, who invoke the term with more heat than light and over-estimate its universal feasibility. Integrated control methods are certainly successful in some areas with important crops but they must be tailored to local conditions. Their use requires not only a constant assessment of the size of the pest population but also carries with it the uncertainty of

Continued on page 388

#### NEWS AND VIEWS

## MARS



From Philip Campbell

THE exploration of Mars by spacecraft began in July 1965, when Mariner 4 passed 12,000 km above its surface and provided the first direct evidence for the existence of craters, a weak magnetic field and a thin atmosphere. Mariners 6 and 7, approaching the planet to within 3,500 km in 1969, indicated that craters were a predominant land form but also revealed the existence of chaotic terrains of irregular ridges and troughs. Carbon dioxide was identified as an important atmospheric constituent, although geologically speaking the planet appeared to be similar to the Moon.

This impression was altered dramatically in 1971 with the arrival of Mariner 9 (the first spacecraft to orbit another planet) and the Soviet Union's Mars 2 and 3 spacecraft. Mars 3 contained a lander that was successfully placed on the martian surface; regrettably, its television camera failed after 20 seconds. Mariner 9 comprehensively mapped the surface and revealed (when dust storms had cleared) not only craters but channels, volcanoes and canyons, all of super-terrestrial proportions. It became obvious that Mars was a more dynamic

planet than had been thought.

Since that time, the planet has been visited by six Soviet and American spacecraft, including the most sophisticated and ambitious of all planetary probes — Vikings 1 and 2. The data provided by the latter orbiters and landers since their arrival in 1976 still occupy the minds of planetologists, as evidenced by the continuing series of regular international colloquia on Mars.

The third of these was held at the California Institute of Technology last August; the full proceedings will appear in the Journal of Geophysical Research next year. Several speakers were invited by Nature to present articles on topics of their choice, concentrating on particular aspects of Mars' solid body and its gaseous envelope, and reflecting to some extent the ground covered at the meeting. The six articles that follow, therefore, are not intended to represent a comprehensive survey of the planet. Nevertheless, it is hoped that they will provide a vivid impression of the radical advance in our understanding over the past few years, and even rekindle some of the excitement that prevailed when the Vikings touched down.

One topic that is barely mentioned here is martian biology. It should be remembered that the Viking landers are sitting on sites where biological activity was thought most likely to be found. But the door on this topic might be said to have closed at the previous colloquium, when it was concluded that the top 10 cm or so of soils at the two lander sites were bereft of organic compounds. A forlorn refrain running through the following articles is the need for further visits to the planet; when such a return materializes, seekers of life will probably want to drill deeper into the surface, where complex molecules may find existence more congenial.

The only future Mars mission known to be under active consideration is the European Space Agency's Mars orbiter, the Kepler mission. If Europe decides to proceed with it, Kepler should be launched towards the end of this decade. Meanwhile, the Viking 1 lander is reactivated at regular intervals to return pictures such as that on the left. But planetologists are trying to salvage what they can from future budgets (as reported in the News section). Will these articles mark the end of this period of unprecedented exploration?

Philip Campbell is a member of the editorial staff of Nature.

#### The geophysics of Mars: whence the Tharsis plateau?

from Sean C. Solomon

THE planet Mars, since the first global views of its surface were sent to Earth by Mariner 9, has been regarded as a key member of the terrestrial planetary family. Intermediate in size between the larger (the Earth and Venus) and the smaller (Mercury and the Moon) bodies, Mars also appears intermediate in the vigour and duration of its tectonic and volcanic activity. Roughly half of its surface is pock-marked with impact craters and basins that formed, by analogy with the Moon, over  $3.8 \times 10^9$ years ago. One third of the surface consists of younger volcanic plains, here and there capped by prominent volcanoes. Major concentrations of volcanoes occur in two broad provinces marked by high surface elevations, positive gravity anomalies and an extended history of surface faulting. The largest of these two provinces, the Tharsis plateau, provided a major focus for new ideas and widespread discussion at the colloquium.

As a backdrop to the discussion of Tharsis, it is well to remember the vast difference between the level of our knowledge of Mars and that of either the Earth or the Moon. We know the planetary mean density and, with less precision, the moments of inertia for Mars, yet we know little of the nature of the Martian mantle or the nature and size of any high-density core. Whether Mars has an internal magnetic field is debatable and is unlikely to be settled by existing data. We have gravity and topographical data of limited coverage and variable accuracy for Mars; we nonetheless do not know the nature and thickness of the martian crust. A relative chronology for major geological units has been derived from crater density measurements; the true ages of martian surface features are not known. The geometry and some relative age information have been obtained for the principal tectonic features, but we do not know the true ages of major tectonic events or the present level of tectonic activity. We are completely ignorant of martian heat flow and of the abundances and distribution of heat sources within the

We are therefore faced with the task of understanding the volcanic and tectonic history of Mars in general, and of Tharsis in particular, without the information on the seismic structure or present thermal regime of the interior that we rely on for similar studies of the Earth and Moon. As a further handicap, that history cannot yet be anchored to the radiometric age of a single martian rock sample. The principal

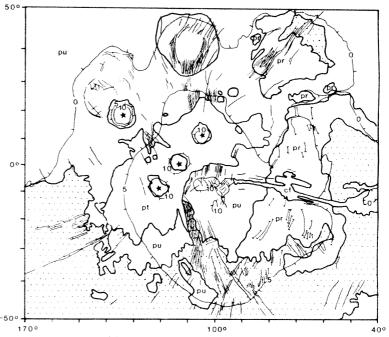
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tools at our disposal in this endeavour are images of the planetary surface, gravity anomalies inferred from the acceleration of orbiting spacecraft, and topographical measurements obtained from photogrammetry and Earth-based radar.

The Tharsis region of Mars (see the figure) is a broad plateau 4,000 km in diameter and standing as much as 10 km above the level of the surrounding terrain<sup>1</sup>. The plateau is covered by relatively young volcanic plains and includes a number of volcanic constructs large and small. There are numerous extensional fault systems and graben that generally predate the youngest volcanic plains and that display a

of most extensional fractures in and near the Tharsis area.

Recent calculations<sup>5,6</sup> of the stresses in the martian lithosphere have called the uplift model, as originally proposed, into serious question. Because the Tharsis region is so large relative to the planetary radius, full spherical calculations including both membrane and bending stresses are necessary to model the response of the martian lithosphere at Tharsis to either doming or loading. An uplift model for Tharsis leads to the prediction<sup>6</sup> of radial compressive features at distances from the plateau centre at which radial graben and extensional features are actually observed.



The Tharsis region of Mars<sup>1,4</sup>. Contours give topographical height in km above Mars datum. Ancient cratered terrain is indicated by light shading. Major volcanic constructs are indicated by heavier shading; the summits of the four largest volcanic shields, indicated by stars, exceed 25 km in elevation. The volcanic plains units include the ridged plains (pr), the younger Tharsis plains (pt) and the undivided plains (pu). Tectonic features include the extensional faults systems (lines), mare ridges (lines with ticks) and the Valles Marineris canyon system (cf).

crudely radial geometry with respect to the centre of the elevated terrain. There are also tectonic features analogous to lunar mare ridges, presumably the result of horizontal compressive stresses, that are more nearly circumferential to the Tharsis plateau.

The traditional explanation<sup>2-4</sup> for the origin and evolution of the Tharsis region is that broad updoming of the martian lithosphere caused by a thermal or chemical anomaly in the mantle led to fracturing, volcanic emplacement of thin plains units, and finally the formation of the youngest large shield volcanoes. Supporting evidence includes the broad topographical height of the Tharsis region, the large elevation of surface units thought to be relatively old from the density of craters and fractures, and the radial trends

As a result, two alternative models for the origin and evolution of Tharsis have recently been put forward. An 'isostatic' model, first proposed by R. J. Phillips and N. H. Sleep<sup>7</sup>, is based on the hypothesis that the topographical height of the Tharsis plateau, and an additional subsurface excess mass required by gravity data, are compensated isostatically by a low density region of the mantle extending several hundred kilometres deep. The low mantle densities may be partly thermal in origin; A. A. Finnerty and R. J. Phillips also described a mechanism whereby the low densities would result from the depletion of lower melting-point basaltic material in the mantle beneath Tharsis.

A contrasting 'flexural' model for Tharsis, proposed by Head and Solomon<sup>8</sup>, is based on an extrapolation to a larger

scale of the geological processes seen in a number of areas on Mars: volcanic loading of the lithosphere, faulting in regions of thin lithosphere or in response to the flexural stresses resulting from large loads, and a concentration of continued volcanic activity in regions of widespread extensional failure. According to this model, much of the topography of Tharsis is due to volcanic construction, and a portion of the topographical load is currently supported by the finite strength of the martian lithosphere.

How can these two contrasting models be distinguished with our existing information on martian structure and evolution? One useful exercise, conducted by J. B. Plescia and R. S. Saunders9 and independently by R. A. DeHon, is to estimate the thickness of the volcanic plains units on the Tharsis plateau from the rim heights of craters partially buried by the plains material. These studies yield estimates of 3-5 km for the maximum thickness of the Tharsis volcanic plains that postdate the heavy impact bombardment of Mars (which probably ended about 3.8 × 109 years ago). Left unanswered by such studies, however, is the question of whether the heavily cratered surface underlying the young volcanic plains in the Tharsis area may itself represent the accumulation of older volcanic units. Thus the total thickness of volcanic material in the Tharsis region is unknown.

The tectonic history of the Tharsis region - the formation of faults of particular types and geometries in particular locations at different stages in martian geological history — holds important clues to the mechanisms responsible for Tharsis. R. S. Saunders and J. B. Plescia described evidence for distinct stages in the development of Tharsisrelated graben and extensional faults; each stage was marked by a different centre of activity. T. R. Watters and T. A. Maxwell addressed the question of the relative ages of mare-type ridges and extensional features where the two types of fault system intersect. Although the evidence that one type of fault is consistently older than the other is equivocal at present, the issue is very important for our understanding of the evolution of Tharsis, since the two types of fault cannot be produced at a given location by a single stress field.

A promising line of research is to combine the information on the history of faulting with calculations of lithospheric stresses as constrained by topographical and gravity data. Calculations by R. J. Willemann and D. L. Turcotte and by R. J. Phillips and W. B. Banerdt have shown that models in which the Tharsis plateau acts as a load on the martian lithosphere predict stresses in broad agreement with observed tectonic features. Phillips and colleagues are finding evidence that older fault systems in the Tharsis area fit best with an 'isostatic' model, while younger fault systems fit better with a 'flexural'

model. The need to keep open the possibility that Tharsis tectonic activity may have also been influenced by globalscale processes was underlined by P. H. Schultz and A. B. Lutz-Garihan, who argued that layered deposits near the martian equator once marked the location of the planet's rotational axis; large-scale polar wander on Mars would have led to global stresses in the martian lithosphere sufficiently large to affect tectonic activity in Tharsis and elsewhere.

If there was a consensus at the colloquium on the nature and evolution of the Tharsis region, it was that none of the simple models for Tharsis topography and tectonics (uplift alone, purely isostatic support, or solely volcanic construction and lithospheric flexure) is likely to be completely adequate. Continued work towards refining and testing these alternatives is clearly warranted. Tasks of high priority for martian geophysical studies in the near future include further elucidation of the chonology of tectonic activity, continued reduction of Viking Orbiter gravity data, new measurements of martian topography with Earth-based radar, and continued development of quantitative stress models to test hypotheses for Tharsis against these new data. For the longer term, our understanding of martian geological evolution will be limited by the large gaps in our information on the planet's surface and interior. Those gaps can be filled only by returning to the planet with new spacecraft experiments.

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#### Volcanism on Mars

from James W. Head III

Single volcanic eruptions, such as the awesome Mount St Helens event, and the frequent spectacular basaltic eruptions on Hawaii, serve to focus our attention on active volcanic processes. Instructive as these local events are, we should not lose sight of the basic reasons why the study of planetary volcanism is important.

If we were to make a list of fundamental things we wanted to know about a planet, two questions would appear high on the list. First, what is the planet made of, and second, how does the planet generate and get rid of its heat? Complete answers to these two fundamental questions require detailed and direct examination of the interior of a planet and a knowledge of how that interior has changed with time. Since it is extremely difficult to examine the interior of a planet directly, we are forced to rely on largely indirect means to answer these questions. The record of planetary volcanism on the surface of a planet is the most important indirect evidence we have.

For example, the record can tell us a lot about the composition and state of the interior. The mere presence of volcanic deposits implies partial melting in the interior and a state of stress in the lithosphere favourable to eruption. In addition, volcanic deposits supply compositional information directly in the form of samples, and indirectly through remote sensing and the interpretation of volcanic morphology. Information on thermal evolution can also be obtained -

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might, for example, compare the volcanic record of the Moon, where 100 per cent of the surface volcanic deposits were emplaced in the first 50 per cent of history, with the Earth, where 70 per cent of the surface volcanic deposits were formed in the last 5 per cent of Solar System history. Clearly, the two bodies had different thermal histories. The individual deposits on a planet and their variation with time are thus important clues to a planet's thermal evolution. Volcanism can also be related to other processes - volcanism and tectonism are often closely interlinked, as in the dramatic Tharsis region of Mars (see review by Solomon), and the volatiles comprising planetary atmospheres must be tied in some fundamental way to planetary outgassing and the history of volcanism.

These general themes raise specific questions about volcanism on a single planet such as Mars. The first task at hand is the identification and characterization of volcanic deposits. The range of morphological features (for example, shields, cones, flows) must be established, and some quantitative measure of these morphologies and estimates of their relative abundances made. At the conference, R. Pike and G. Clow (US Geological Survey) reported quantitative measurements of martian volcanoes and comparisons with similar structures on Earth, concluding that there is good evidence for three separate classes of volcano on Mars and that most martian shields differ substantially from terrestrial shields. These data are of the utmost importance for the characterization and



Oblique view of the massive martian shield volcano Olympus Mons. Recent topographical data (S. Wu et al. 3rd int. Colloq. on Mars, 287-289), indicate that the peak rises some 26 km above Mars datum, and that the caldera complex is about 80 km in diameter. A scarp up to 8 km in height surrounds the 600 km wide shield.

understanding of eruption conditions. C.A. Wood (NASA Johnson Space Center) compared calderas on Mars and Earth and noted the relatively low abundance on Mars, attributing it to the lack of both plate tectonic subduction zones and associated abundant silicic volcanism on Mars. S. Wu and co-workers (USGS) presented a topographical map of the massive Olympus Mons shield volcano (see figure), and E. Morris (USGS) described much new photographic data on the structure of this shield, the nature of its basal scarp (rising 4-8 km above the surrounding plain), and on the distribution and characteristics of the thin, lowviscosity flank flows. M. Zeitner (University of Washington) presented evidence that some of the circular craters associated with graben on Mars may represent maar-type eruptions. A study of the distribution and properties of small cones on Mars (H. Frey and M. Jarosewich, NASA Goddard Space Flight Center) provided data to help distinguish between origins as cinder cones, pseudocraters and pingoes. Variations within and between regions suggest multiple origins for the martian examples. P. Spudis and R. Greeley (Arizona State University) des-

cribed the geology of Tyrrhena Patera, an ancient central vent volcano in the cratered terrain, and interpreted its structure as representing a style of martian pyroclastic volcanism that may have been more common, but not abundant, in early martian history. G. Schaber, K. Tanaka (USGS) and J. Harmon (National Astronomy and Ionosphere Center, Arecibo) reported topographical data from Earth-based radar observations and several new volcanotectonic collapse features and previously unmapped lava flows within Syrtis Major Planitia. The topographical data show a dramatic 5 km rise near the edge of Syrtis Major, rather than the gently sloping surface previously thought to represent the topography. The authors suggest that the elevation of the entire Syrtis Major feature, the previously reported absence of any basin rim mountains or rim ejecta, the rapid decrease in elevation into Isidis Planitia, and the absence of any strong gravity anomaly centered on Syrtis Major, all indicate that the early geological history of this feature does not include the formation of an impact basin as postulated earlier. The feature appears to consist instead of a vast volcanic sequence whose source fissures

(probably concentric to the Isidis Basin), and subsequent extrusive episodes are related to crustal fracturing or fracture rejuvenation following the Isidis impact event early in martian history.

Characterization also requires data on the composition and physical properties of these units. Here remote sensing plays an extremely important role. On the Moon, we have nine sample return sites, yet global characterization of volcanic deposits is still mainly derived from remote sensing data. On Mars we have no sample return sites so we must rely on remote sensing data even more. Remote sensing can provide data on the composition and physical properties of surface materials and can also be used to distinguish, characterize and map surface units. H. Kieffer (USGS) reported a series of global maps of Mars data that have been compiled to similar scales; they will be of great importance in global mapping of volcanic deposits. J. Zimbelman and R. Greeley (Arizona State University) studied the surface properties of the Ascraeus Mons shield using remote sensing data and showed that its surface is characterized by a uniform average particle size, arguing against the presence of localized ash deposits. T. McCord (University of Hawaii) and co-workers used Viking approach images to define global surface units and a range of other information -Viking orbital, Earth-based telescopic spectral and albedo data - to characterize these units. The maps emphasize the composition of surface units and differ from traditional geological maps in that they include condensates and eolian deposits as well as bedrock units. The approach holds great promise for distinguishing and characterizing volcanic units on Mars

Another promising area is the investigation of Earth samples with similar spectral properties to surface units on Mars. D. Evans (University of Washington), R. Singer (University of Hawaii) and co-workers showed that certain weathered basalt tephras and a basalt coating from Hawaiian rocks have similar spectral characteristics to surface units at Viking Lander 1 and to some global units. The final results for the chemical composition of martian fine-grained soil from the X-ray fluorescence spectrometers at the Viking landing sites [B. Clark (Martin Marietta) and A. Baird (Pomona College)] can be interpreted as representing a primary crustal rock type, a mafic flood basalt rich in pyroxene.

An understanding of the range of eruption conditions represented by the deposits and their significance in terms of magma types, volatiles and the state of stress in the lithosphere can be approached by looking for direct morphological analogy with features on Earth. This approach may help to establish the volcanic nature of major landforms, such as the shields, but may be quite misleading for understanding the eruption conditions

leading to specific features on Mars. We know, for example, that gravity and atmospheric density differ between Earth and Mars and can markedly influence eruption conditions and resulting landforms. It is important, therefore, to consider the process of ascent and eruption of magma on Mars from first principles and to develop from these theoretical analyses predictions of the types of volcanic landforms that might be produced in the martian lithospheric and atmospheric environment. L. Wilson (University of Lancaster) and J. Head (Brown University) presented theoretical analyses of the behaviour of both volatile-free magma and magma containing volatiles and the resulting deposits and landforms. A specific example interpreted as a relatively young pyroclastic air-fall deposit on the flanks of Hecates Tholus was described by P. Mouginis-Mark, (Brown University) and Wilson and Head. From deposit characteristics and models of explosive eruptions, possible eruption conditions for this unique deposit were outlined. Although ash deposits had been thought of as candidates for various units on Mars, this appears to be the first identification of a specific deposit from a source region.

Armed with data on the characteristics of deposits and the range of eruption conditions, it would be useful to know the distribution and volume of volcanic deposits in space and time. Greeley and Spudis (Rev. Geophys. Space Phys. 19; 13-41, 1981) have recently reviewed the field and it is obvious that we have a long way to go to identify and characterize deposits and to relate them to the impact crater chronology. Progress in this area was reported by P. Francis (Lunar and Planetary Institute, Houston) and C. Wood (NASA Johnson Space Center), who reviewed evidence that silicic pyroclastic volcanism on Mars is unlikely to be a major process.

J. Plescia and S. Saunders (Jet Propulsion Laboratory, Pasadena) and D. Scott and K. Tanaka (USGS) have recently compiled stratigraphies of the volcanic deposits in the Tharsis region of Mars. Although information is accumulating, our understanding of the significance of these data for the thermal evolution of Mars is hampered by a lack of returned samples and of a knowledge of absolute ages. L. Martin (Lowell Observatory) reviewed evidence for possible volcanic activity on Mars during the Viking mission. Despite the excellent geological maps of the USGS, Dave Scott (USGS) showed that many volcanoes and volcanotectonic structures in the western hemisphere of Mars could be mapped using newer Viking Orbiter images, particularly in the older parts of the martian crust.

Where do we stand in terms of achieving the basic objectives outlined above? On the basis of the conference results, it is obvious that much more work of the type reported at the conference is required to characterize the martian volcanic record. We need to integrate the full range of remote sensing data in order to complement surface morphology with information on composition and physical properties. Some of this information is available, but much remains to be collected from Earth-based observations and from spacecraft near, and on, Mars.

Some of the basic theory of magma ascent and eruption on Mars has been worked out but much work needs to be done to compare present deposits with the theoretical predictions. This will help to establish the full range of eruption conditions and the implications for magma composition and the role of volatiles from both deep and shallow sources. The dis-

tribution of volcanic units in space and time is becoming better known but is far from a level which can constrain thermal evolution. Continued comprehensive mapping is required. A major problem is the nature and level of volcanic activity in early martian history during the time of high impact flux. Even with such information in hand, an absolute chronology is required before the thermal evolution of Mars can be understood.

In summary, progress towards these goals can be made with existing data, but major strides in these areas of fundamental importance to the evolution of planets requires new data of a variety of types, data obtainable by returning to Mars with properly instrumented spacecraft.

#### Martian geology

from Michael H. Carr

In addition to volcanism a wide range of geological topics, including channel formation, subsurface migration of water, simulation of martian ejecta patterns and sedimentation at the poles, were discussed at the colloquium. Surficial deposits such as the regolith and the relatively mobile eolian cover received most attention, possibly because a wealth of new experimental and observational data now allows them to be treated in quantitative terms.

Several papers were concerned with weathering. It has been suggested that UV light has an important role in chemical breakdown of materials on the martian surface but new data (M.C. Booth and R.V. Morris, Johnson Space Center) show that under martian conditions, with modest partial pressures of H<sub>2</sub>O, oxidation of magnetite, dehydration of goethite and breakdown of silicates take place at rates independent of UV radiation. Other experiments (T.R. Blackburn) show that breakdown of sulphides to sulphate can also occur without irradiation but breakdown is more rapid in samples irradiated by UV. R.L. Huguenin (University of Massachusetts) expanded upon his frost weathering model in which dissociation of adsorbed water on mineral grains and migration of hydrogen ions to lattice defects allow recombination of the OH- radicals left on the surface to produce  $H_2O_2$  — possibly the active oxidant needed to explain the Viking biology results. The reactions he proposes are all exothermic and do not depend on UV radiation, suggesting the role of UV in martian weathering is less important than previously thought.

The precise nature of the weathering products remains uncertain. Attempts to

Michael H. Carr is in the US Geological Survey, Menlo Park, California. match telescopic reflection spectra of Mars bright regions with various terrestrial materials have shown the Mars spectra to be inconsistent with ferric oxides or ironbearing clays. The best fit is with palagonite, a largely amorphous reaction product of basaltic lava with water which had been previously suggested by several workers, including the Viking Inorganic Analysis Team, to be a possible Mars surface material. However, A. Bonin reported work at the Hebrew University, Israel, on simulation of the results of the Viking biology experiment. He showed that Fe-rich clays can catalyse decarboxylation and/or oxidation of organic acids and was able to reproduce accurately the Viking labelled release results. Fe-rich clays were also used successfuly to simulate the gas exchange and pyrolitic release experiments.

The distribution and physical nature of the surface debris attracted attention from a number of workers. A map of the global distribution of different colour units identified on a planet-wide plot of red albedo versus violet albedo was derived from the Viking Orbiter approach photography (McCord et al., University of Hawaii). The differences were attributed to rock-soil mixes and condensates and tentative correlations of the R-V units with units identified from telescopic spectra, which mostly resemble those of oxidized basalts, were made. A related, but more detailed, study (E.L. Strickland, Washington University) correlated colour variations seen in orbital pictures of the Chryse region with colour variations observed around Viking Lander 1, and constructed an elaborate stratigraphical sequence to explain their apparent superpositions.

Several papers were concerned with eolian processes. R.E. Arvidson of Washington University pointed out that

despite an active eolian regime on the planet, the survival of craters on relatively ancient surfaces implies that bedrock erosion rates are extremely small (< 10<sup>-7</sup> cm yr-1). The wind-induced changes observed must be due almost entirely to remobilization of relatively loose, friable debris. Wind tunnel experiments (R. Greeley et al., Arizona State University) using a number of different sands and targets, which simulate wind abrasion on Mars, showed that for all conditions, abrasion rates were several orders of magnitude higher than those estimated for Mars. The results can be reconciled. Greeley suggests, if sand on Mars consists mainly of easily destroyed aggregates rather than discrete mineral grains. The aggregates probably consist of relatively soft weathered products, which disintegrate easily and have limited abrasive capability. They are nevertheless capable of saltating and forming dunes which are numerous especially at high latitudes. A somewhat different view of eolian erosion (J. McCauley, M. Grolier and C. Breed, US Geological Survey; F. El Baz, Smithsonian Institution) is that it has taken place extensively in the high northern latitudes of Mars to produce the so-called knobby terrain. On the basis of comparisons with landforms in the deserts of south-west Egypt, they propose that the old cratered terrain was initially eroded by fluvial processes and then the interfluves eroded away by eolian and mass wasting processes to leave the knobby remnants that we now observe.

Apart from volcanic studies and several papers on the Tharsis bulge discussed above, discussion of the bedrock geology was restricted to relatively few topics. Channels remain as controversial as when they were discovered a decade ago, and following papers by H. Masursky, M. Carr and B. Lucchitta (USGS), a lively debate took place on the origin of large channels. with water, ice and wind each receiving support. On two points a consensus is emerging; the large outflow channels (see figure on facing page) have a wide range of ages, and the branching valley networks are mostly very ancient. As to origins, it appears that most large channels are complex and a variety of processes, including catastrophic flooding, glacial scour, wind erosion and later filling by lava, may have contributed to their final form. In a related topic R. Huguenin and S. Clifford (University of Massachusetts) elaborated on their previous suggestion that water sources (oases) exist in the Solis Planum and Hellespontus regions. They suggest that continual loss of water from the regolith to the atmosphere in the equatorial regions causes a slow migration of water from the poles to the equator in the megaregolith below the permafrost. The rates are extremely slow and significant only over millions of years. The ground water is replenished by condensation of water from the atmosphere onto the poles.

Finally, several papers dealt with impact craters and what they might tell us of the nature of the martian surface. In almost all areas more than 20 per cent of fresh impact craters have central pits (W. Hale and J. Head, Brown University). These have been previously ascribed to volatiles in the surface and their ubiquity may indicate the omnipresence of ground ice. In a more elaborate study (K. Blasius and J. Cutts. Science Applications), the distribution of craters with different types of ejecta patterns was mapped and showed a complex dependence of ejecta types on geological unit, latitude and possibly other factors. Experimental simulations of martian ejecta patterns have been conducted (P. Schultz and D. Gault, Ames Research Center) and demonstrate that ejecta ramparts closely resembling those on

Mars can be reproduced by impacts into loose debris in the presence of a moderate atmospheric pressure.

Thus, in contrast to the previous Mars colloquia, emphasis of the geology papers was on surficial processes. This may simply reflect the ease with which the relevant available data can be manipulated. The infrared, colour and chemical data, upon which much of the interpretation of surficial processes rests, are readily handled in the computer. In contrast, interpretation of surface morphology, which more closely reflects the bedrock geology, largely depends on the much longer process of direct visual inspection. Analysis of the enormous volume of Viking Orbiter images to improve our knowledge of the geological evolution of the planet will clearly take many years.

## Mars climate change: where are the petroglyphs?

from Fraser P. Fanale

At one point in the colloquium it was pointed out that petroglyphs illustrating both abundant game and well preserved fragments of ostrich eggs thousands of years old could be found in almost rain-free portions of the Egyptian desert. These were considered indicative of a formerly wetter climate in that currently extremely arid region<sup>1</sup>. By analogy, most of the papers directly aimed at the planet Mars seemed to seek some natural 'inscription' of morphology on the martian surface that might indicate a prior warmer wetter period (or, as we say, a more 'paradisical' period) in martian history. To be currently recognized, such an inscription or morphology would have to be large enough to be identifiable in available Viking Orbiter images.

The candidates most often discussed are the various channels of different sizes and detailed geometry<sup>2-4</sup>. The huge 'outflow' channels (see the figure) are spectacular but seem unconvincing as indicators of a very different past climate at the Mars surface. They lack tributaries and seem to spring at full size from what appear to be collapse features. These and other observations suggest that they burst forth from subsurface water reservoirs dammed up by permafrost<sup>5</sup>. Thus their relationship to global conditions in the Mars surface megaenvironment or at the atmosphere-surface interface may be subtle and indirect, or nonexistent. They could probably form today.

The so-called 'runoff' channels are valleys or gullies which are shorter, but

have well developed tributaries, form regional networks and are more suggestive of regional slow erosion by surface runoff<sup>6</sup>. When this geometry is examined in detail, however, it resembles that caused by headward 'sapping' processes involving subsurface water more than that expected from ordinary rainfall7. Nonetheless, there are two aspects of these 'runoff' channels (evidently 'network' channels would be a safer term) that persist in indicating global climate change. First, the network channels are widely believed to occur only in the oldest terrain on Mars6. Second, they are globally distributed within that terrain. The global distribution suggests that rainfall may have had some role even if sapping was involved as well. Moreover the restriction to the oldest (4.0 billion years?) terrain may be in harmony with theories suggesting that global conditions involving high atmospheric Pco, (ref.8) or a reducing atmosphere would have to be sustained by an atypically high degassing rate, possibly associated with accretion9,10, and that such conditions could not prevail for long in the face of planetary hydrogen loss and removal of CO<sub>2</sub> to form rock-attacking acid. It has been argued, however, that many variables (other than age) may determine channel distribution and that the restriction of any channel type to earliest Mars history has yet to be proved11, while others argue a dominant role for ice12,13 or wind14 in carving the channels. Although a role for wind and ice in modifying the channel morphology is readily conceded, water seems to be required to explain some channel characteristics such as their reliable and familiar tendency to flow

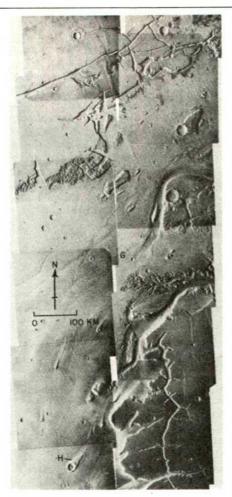
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Besides the channels we have various morphologies indicative of the presence of subsurface H2O ice or even liquid water. One is the so-called 'thermokarst' terrain which resembles the surface distortion and cracking patterns observed in regions of hard frozen (ice-filled) permafrost on Earth<sup>16,17</sup>. Subsurface H<sub>2</sub>O may also be indicated by 'nonlunar' or cohesive flow characteristics in crater ejecta. Most investigators concede that subsurface volatiles are involved in producing such ejecta but disagree as to how many separate categories of 'sloppy' throwout there are, whether or not some types are latitude dependent18,19 and which types require a specific role for water ice, liquid water, adsorbed CO2 and atmospheric CO2. A combined approach which studies the dependence of throwout morphology on crater size, target age and target material, together with experimental cratering in icy and muddy targets - some of which are multilayered - may allow these variables eventually to be sorted out20.

Yet another 'petroglyph' is the 'layered terrain' developed near the edge of the north cap and discovered by the Mariner 1971 mission. Since its discovery, a strong connection between the Mars layered terrain and oscillatory astronomically driven climate change has been indicated21,22. Such a connection, in turn, would conceptually link climate change on Mars to astronomically driven glacial cycles on Earth. Current quantitative models23-26 could be summarized as follows: as the obliquity (angle between rotation axis and normal to the ecliptic) of Mars increases from its present value ( $\sim$ 24°) to its maximum (37°), the increased facing of the polar regions to the Sun will cause warming of these critical regions.

Although the large north cap is now known to be H2O ice, not CO2, and although the CO<sub>2</sub> south residual cap is almost trivial as a CO, reservoir, the atmospheric pressures could possibly double or even triple as a result, owing to desorption of CO2 from large circumpolar and high latitude deposits of weathering products and pulverized igneous rock. When the obliquity falls far below its present value (to  $\sim 11^{\circ}$ ) then the poles cool much more than the regolith which exists at a variety of depths and latitudes, and which experiences a far more attenuated thermal 'wave' (the equatorial regolith even warms). Hence the greatly cooling polar regions will 'cryopump' most of the CO, from the vast 'ocean' of adsorbed CO, in the regolith, resulting in the sudden appearance of a huge CO2 polar cap (as the north cap was originally thought to be), seemingly from nowhere. In the process the atmospheric pressure falls to less than 0.5 mbar.

Thus in the models, the atmospheric pressure varies by a factor of 20 to 100 while giant CO2 caps appear and disappear with the clock-like precision of the



Viking photomosaic of a portion of Kasei Vallis centred at approximately latitude 20°N, longitude 75°W. Outflow features such as streamline hills (H) and grooves (G) occupy the channel floor, but valley sides, tributary canyons and inner channels of the valley floor show pronounced structural control by fracture systems.

From Baker, V.R. & Kochel, R.C. J. geophys. Res. 84, 7961 (1981).

obliquity cycle - a time scale of 105 years. Intuitively it would seem that the harmonic changes in the atmosphere's ability to transport dust and the concurrent variations in cap extent would provide ample means of producing the layered

Current efforts to work out the mechanics of this process take advantage of the fact that the current pressure is just barely enough to raise dust. If pressure decreased by a few millibars, then dust storms would be rare; but if this pressure increased by a factor of two, they might be almost continuous25

Going back further in martian history it is thought that the obliquity cycle was of still greater amplitude before development of the huge Tharsis volcanic complex and associated gravity anomaly. This is because Tharsis near the equator actually stabilizes the planet's axial wobble27; thus the effects discussed would be even more dramatic before Tharsis. Yet investigators keep insisting that the layered terrain is a feature of the latter portion of Mars' history. One solution offered at the meeting is that Tharsis was not formed at the equator, but rather flipped the planet over, causing global wandering of the poles. This could have left several prior 'layered terrains' stranded as outliers about the planet and these could, following erosion, be recognizable only as some sort of blanketing feature of which there are many28. Other solutions involve a steadystate system in which layers are destroyed at the base of the piles as fast as new ones are laid down29. All this provides a marvellous example of comparative planetology since the Earth's climate is affected by the same astronomical mechanism with similar periods but lower intrinsic amplitude and seemingly more complex feedback mechanisms than for Mars (see ref. 30).

The regolith-atmosphere-cap models show that even the more violent regolithatmosphere-cap interaction before Tharsis would, by itself, be incapable of creating a sufficiently profound change in atmospheric CO, pressure or composition to produce a stable greenhouse system that could accommodate rainfall despite the lower energy output of the ancient Sun10,31. Thus a magic additional ingredient that seemingly stabilized a more clement environment early in the history of Mars and produced the infamous channel 'networks' remains unidentified.

In summary, one is tempted to divide

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Mars' climate history into three parts. The most recent part would be post-Tharsis (the past about 1 billion years) when obliquity variations force CO2 exchange between the two large CO<sub>2</sub> reservoirs, the regolith and periodically appearing caps, through the conduit of the atmosphere. During this process the atmospheric pressure varies harmonically by more than 20-fold. The previous epoch (4.0 to  $1.0 \times 1.0^{99}$  years?) saw the very same processes dominating, but with larger amplitude due to the absence of Tharsis as a stabilizing influence. Also other 'nonharmonic' or 'irreversible' trends (such as gradual changes in regolith mass, amount of

exchangeable CO<sub>2</sub>, solar constant) may have changed the quantitative aspects of the operation of the three-part system. Finally we have the 'networks' with their fascinating suggestions of an epoch of more clement conditions in earliest martian history. These may require feedback mechanisms such as gas or dust greenhousing or greater atmospheric heat transport to the poles, in addition to the CO<sub>2</sub> exchange. In any event, the networks are found (only) long ago and in another land. The issue of whether or not the wench is truly dead is one best left to the biologists who were not represented among the speakers at the colloquium.

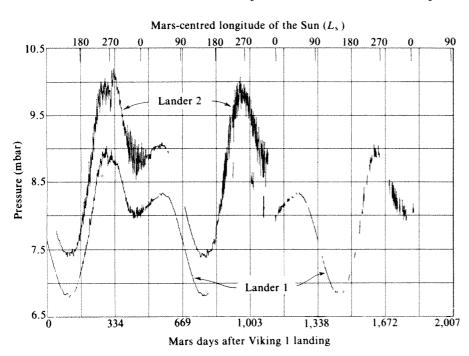
#### The martian lower atmosphere

from Conway B. Leovy

ALMOST three martian years (more than five Earth years) of atmospheric pressure data at two Viking Lander sites have provided a remarkably detailed picture of the weather and climate on Mars. These data, presented at the colloquium by J.E. Tillman<sup>1</sup>, revealed the seasonal shifting of mass between the carbon dioxide polar ice caps and the atmosphere, global circulation changes associated with major dust storms, travelling waves in middle latitudes and intense atmospheric tides driven by absorption of solar radiation in the dust-laden atmosphere.

The seasonal cycling of pressure, involving as much as 30 per cent of the mass of the atmosphere, produces a massive atmos-

pheric movement back-and-forth between the two polar regions, and is indicative of the sensitivity of atmospheric mass to the polar cap radiation balance<sup>2</sup>. Narumi has shown that radiation balance models can account well for the seasonal pressure variations if allowance is made for seasonally varying polar clouds3. The radiation balance is itself highly sensitive to the albedo of the polar caps, and, in a detailed analysis of Viking Orbiter data, D. Paige4 has shown how differences in albedos of the two caps can explain differences in their seasonal behaviour. At the end of summer, there is a small residual cap at each pole: the northern residual is H<sub>2</sub>O ice while the southern is CO<sub>2</sub> ice.



Daily averaged pressures at the Viking Landers. The ordinate interval is the number of Mars days in a Mars year.  $L_s$  values (top) correspond to season: 0, northern spring equinox; 90, northern summer solstice; 180, northern autumn equinox; 270, northern winter solstice. Gaps correspond to missing data.

Paige attributes this difference to the relatively high albedo of the south cap during summer. The seasonal pattern of dust storms provides a likely explanation for cap albedo differences, dust storms being most frequent and widespread during the season of CO<sub>2</sub> condensation on the northern cap. Tillman's data pose a difficulty for this view, however, since they show little or no year-to-year variation in the rates of condensation or sublimation of CO<sub>2</sub> polar ice despite apparently large year-to-year differences in the behaviour of dust storms.

A particularly spectacular global dust storm occurred during Viking's first year. Although several explanations have been proposed for the origin of these global storms, there has been no fully satisfactory quantitative theory of their life cycle, incorporating both storm birth and death. A step in this direction may have been taken by H. Houben<sup>5</sup> who used a highly simplified atmospheric circulation model to show that global-scale atmospheric disturbances can grow if the dust content is non-uniformly distributed and heating is sufficiently intense, but no global-scale disturbances can grow if the dust is uniformly distributed. Since the storms tend to produce a relatively uniform distribution of dust, this could account for storm decay without regeneration of new storms. Further study will be needed to determine whether Houben's model is applicable to the real martian atmosphere.

In another modelling study of dust storms, R. Haberle showed how dust raised in a narrow latitudinal belt, such as the southern summer subtropics, could cause a dramatic intensification and expansion of the thermally driven, zonally symmetric tropical circulation, the so-called Hadley circulation, in both hemispheres6. Tillman's data together with infrared data from the Viking Orbiters showed that such an expansion and intensification of the Hadley circulation actually did occur during the global dust storm observed by Viking. These observations have farreaching implications for the relationships governing the latitudinal extent and intensity of Hadley circulations, in particular, how Hadley circulations depend on such governing parameters as planetary radius, rotation rate and heating intensity. The relationships seem to be applicable not only to the circulations of the 'sister' planets Mars and Earth, but also to the more exotic circulations at the cloud-top levels of Venus and Jupiter.

Martian atmospheric data are also helping to broaden and generalize existing theories of large-scale atmospheric waves. The travelling mid-latitude waves measured by the pressure, wind and temperature sensors at the Viking Lander sites (see the figure) have longer wavelengths

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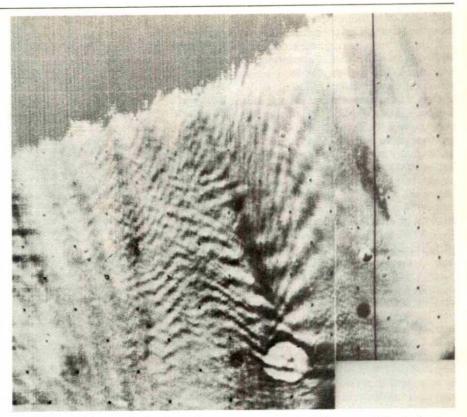
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and are much more regular than their counterparts, the familiar travelling cyclones and anticyclones of terrestrial mid-latitudes. J. Barnes presented results of efforts to understand the wave properties theoretically7. The high martian static stability and the zonally aligned topography both tend to stabilize these disturbances relative to their terrestrial counterparts, and may be the causes of the great observed regularity. More generally, the scales of the observed waves and those on the other planets seem to fit in with a general scaling for planetary waves similar to that for the Hadley circulations. (See the photograph).

Aspects of the climatic history of Mars are discussed in detail in the preceding article by Fanale. The regolith and CO2 polar ice caps seem to have had crucial roles in buffering atmospheric CO, pressure since the time of regolith formation. The regolith and polar caps also serve as reversible reservoirs of H<sub>2</sub>O. Viking Orbiter measurements of atmospheric water vapour may provide an indication of the relative roles of seasonal adsorption in the regolith and condensation in the polar cap, but the interpretation of these data remains controversial. B. Jakosky and C. Farmer<sup>8</sup> believe that the seasonal variations of H2O are dominated by adsorption, whereas D. Davies and L. Wainio consider exchange with the polar cap to be more important9.

An important new result relevant to the earliest period of atmospheric history was a careful recalculation of the escape flux of nitrogen by J. Fox and A. Dalgarno10. Viking measurements showed an enrichment of 15N with respect to 14N by a factor of 1.7. Fox and Dalgarno were able to show that their calculated escape is consistent with this enrichment and an initial N2 endowment of as few as ten 22 N, molecules per cm2, much more than the current value but substantially lower than the early post-Viking estimate of McElroy et al. 11. The escape flux predicted by Fox and Dalgarno is sensitive to assumed details of the ionospheric structure which have not yet been measured, and to certain other assumptions. If the assumptions are correct, and if the N2/CO2 ratio of Mars is in line with that of the Earth and Venus, the Fox-Dalgarno calculation suggests a total evolved CO, mass equivalent to as little as 50 mbar, but it does not demand such a low





Mariner 9 photograph showing a lee wave system produced by crater Milankovic, a 100-km diameter crater at 53°N, 148°W. The lee waves extend 800 km downstream to the evening terminator with a wavelength of about 60 km.

## Exploration of the upper atmosphere and ionosphere of Mars

from C.T. Russell and A.F. Nagy

STRATEGIES for the exploration of the Solar System and its individual planets have been prepared by a number of different committees and boards. In recent years, the major factor restraining the pursuit of such plans has not been lack of suitable technology but of money or, more specifically, the willingness to spend that money on any particular objective. To establish a logical strategy, the sophistication of the planned investigation and the accessibility of the planet play important roles. Whether sophistication is measured in terms of the type of mission flyby, orbiter, lander, rover or manned or in terms of scientific objectives, the ranking of a particular mission remains roughly the same. Again, whether the accessibility of a planet is measured in terms of the required launch vehicle capability or in engineering considerations such as thermal and power requirements and mission lifetime or in available telemetry rate, one will obtain roughly the same ranking.

The cost of a mission depends on both sophistication and accessibility. For roughly the same cost one could choose to fly a very sophisticated mission to the Moon, a modest mission to Jupiter or a very simple mission to Pluto. The total money available for the planetary program, is, however, limited and choices have to be made. It is sensible to choose a mixture of sophisticated and simple missions, because detailed data obtained from the more accessible bodies can help us to understand the behaviour of the more inaccessible objects, and comparisons between less sophisticated and more fragmented data obtained about a group of planets can be used to learn more about the generalities of planetary evolution. This approach is usually adopted because budgets are tight, and mission planners must seek missions that provide new and exciting data at the minimum cost. However, there are exceptions to this

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approach, two of the most major ones being martian and lunar exploration.

Consider for a moment the strategy for exploring a single planet. It appears logical that the initial step should be a reconnaisance with a flyby followed by an orbiter. The flyby would provide snapshots of some characteristics, and the orbiter would give global data over a longer period of time. The instrumentation and objectives of the orbiter will depend on the results of the flyby. It is not obvious that a single flyby or a single orbiter can do the entire job — orbits suited for one objective may be unsuitable for another, for example. Orbiters certainly provide increased observation time, but may also make more sophisticated and/or global measurements possible. Landers provide access to the surface and a longer period for observation and analysis, although at only one site. Sample return provides access to yet more sophisticated analysis as well as allowing longer time for analysis.

Both the United States and the Soviet Union have orbited spacecraft around Mars. The US missions were directed principally towards surface, photogeology and low altitude atmospheric science objectives. The Soviet missions looked at the solar wind interaction with Mars but telemetered little data and had relatively high minimum altitudes that did not penetrate the upper atmosphere and ionosphere of Mars. Hence there is only very limited information on the nature of the upper atmosphere and ionosphere of Mars, little geophysical or geochemical data, only a rough idea of the topography and an essentially unresolved controversy on the existence of an intrinsic planetary magnetic field. Furthermore, the proper global geochemical and geophysical data to plan an intelligent surface exploration strategy are not currently available. The US skipped a stage of exploration in order to be first to return data from the surface. The US and European scientific communities have become aware of this void and are coming forward with proposals to fill the gap. An ESA mission, KEPLER, has been proposed but unfortunately it competes with the Polar Orbiting Lunar Observatory (POLO), also in the planning process at ESA, which is designed to fill the analogous gap in our lunar knowledge. In the US, a Pioneer class Mars Orbiter mission, in many ways similar to KEPLER, has been discussed, but the chances for such a mission are very uncertain given the present tight financial situation at NASA.

In view of this lack of data there was little discussion of martian aeronomy or the solar wind interaction at the conference. J.A. Slavin (UCLA) addressed the existence of a planetary magnetic field. The few cases in which the Soviet Mars orbiters were claimed to enter either a martian magnetosphere or magnetotail are subject to varying interpretations. How far does the bow shock stand off of the planet and what size object would be necessary to create a



Photograph of Mars taken from a distance of 419,000 km by Viking 2. Near the top is the volcano Ascraeus Mons, with a white water-ice cloud to the north-west. The Valles Marineris is visible below the volcano, and the impact crater of the Argyre Basin, covered with winter frost, at the bottom of the photograph. The picture below shows the horizon of Mars with clouds, thought to be crystals of CO<sub>2</sub>, 25–40 km above the surface. Taken 11 July 1976 by Viking Orbiter 1.



bow shock in this position? Slavin showed that a size slightly larger than the planet plus its ionosphere is necessary and thus he feels there is a weak intrinsic field with a moment of about  $1.4 \times 10^{22} \, \mathrm{G}$  cm<sup>-3</sup>, about 5,000 times smaller than the Earth's moment. Examining available ionospheric data, principally from radio occultations, he finds no evidence for a Venus-type interaction. On the other hand, at altitudes above the ionosphere there are similarities in that heavy ions are found to be carried

away by solar wind from both planets.

The lack of martian aeronomical data is particularly intriguing to planetary scientists because of the contrasts between Venus, Earth and Mars. Earth and Mars have similar rotation periods. Venus and the Earth have similar gravitational fields. Mars and Venus have similar atmospheric composition. Venus has no - or at least no detectable - planetary magnetic field while Mars has at most a weak one. In view of these contrasts, will the martian thermospheric temperature be controlled by solar EUV like the Earth's or be rather insensitive to solar EUV like that of Venus? Does Mars have a strong upper atmospheric wind system like Venus?

The ionosphere of Mars is similarly an enigma. Is there a solar wind source of heat as on Venus? What maintains the nightside ionosphere revealed by radio occultation? The terrestrial ionosphere is partially maintained at night by the large reservoir in the magnetosphere that fills with plasma during the day and empties at night. On Venus the night ionosphere is maintained by rapid flows from the dayside and by energetic particle bombardment from the wake of Venus. Finally, the chemistry of the martian ionosphere is a virtual unknown. The neutral atmospheric composition and hence the ionospheric composition are expected to be similar to that of Venus. However, the reaction rates are temperature and velocity dependent and therefore quite a few surprises may be in store.

Venus was found to have an extended non-thermal hydrogen and oxygen corona even though it has a very low exospheric temperature; the presence of this corona is due to dissociative recombination and charge-exchange reactions. On the other hand the Earth has an extensive hydrogen corona caused by the Jean's escape flux and charge-exchange processes, but it does not have a significant oxygen corona. The solar wind is deflected by the terrestrial magnetosphere so there is little significant interaction with the geocorona, but in the case of Venus, with no intrinsic field, the situation is very different, in that there is significant absorption by the corona through ionization in and charge-exchange with the flowing solar wind. In this regard Venus acts very much like a comet. Does Mars behave the same way, or is its corona shielded from the solar wind by a small magnetosphere?

These questions and many more must wait until we get back to a logical series of explorations, for despite the sophistication of some of our knowledge about Mars we are completely ignorant about some of its basic features. Our best hope is ESA's KEPLER or NASA's Pioneer Mars Orbiter missions. The realization of either of these missions would provide a big step forwards in our knowledge of Mars, as well as exposing many areas of ignorance to stimulate further progression in our exploration of the planets.

# REVIEW ARTICLE

# The role of gene dosage and genetic transpositions in carcinogenesis

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Analysis of chromosome translocations associated with B-cell derived tumours and studies of chromosome-15 trisomy in murine leukaemia support the theory, emerging from recent work on tumour viruses, that cancer can be associated with DNA rearrangements which result in the increased expression of normal cellular genes.

IN a recent review on the origin of human cancers, Cairns<sup>1</sup> suggested that most human cancers result from genetic transpositions, rather than mutations. The purpose of the present discussion is to review some of the evidence dealing with the role of specific translocations in the genesis of certain human and animal tumours. Recent findings on mouse plasmacytoma and human Burkitt's lymphoma suggest that the translocations frequently associated with these tumours may act through the activation of specific gene(s) that regulate the growth and differentiation of the lymphocyte-plasmacyte series. It is suggested that translocation of the relevant chromosome segment to an immunoglobulin determinant-carrying region brings the gene(s) under the influence of a highly active cellular promoter, leading to increased expression of the gene product. It will be further argued that increased gene expression is the cause of murine leukaemia associated with trisomy, although in that case the gene may also be altered by mutation or viral promoter insertion. The general theme, that cell transformation is the result of an increase in the expression (or functional activity) of a normal cellular product, will be considered in the light of existing knowledge about the action of virally transmitted, cell-derived oncogenes.

#### Viral oncogenes

Retroviruses: The rapid expansion of viral oncology during the 1960s was considerably motivated by the expectation that specific, virally coded genes determine the synthesis of transforming proteins. This turned out to be true in one sense, but the situation is quite different from what was expected. In several cases, the transforming proteins associated with the directly acting retroviruses are similar to normal cellular constituents, amplified in amount and/or changed in functional activity. All viruses of this category share the following properties:

- First, they can induce tumours in susceptible hosts in vivo after short latency periods.
- Second, they transform cells in vitro.
- ◆ Third, the viral genome carries sequences that appear to have been derived from the normal host genome ('oncogenes'). In most cases, they are inserts that replace large portions of the viral genome. Consequently, the virus becomes defective and requires a helper for replication. Retrovirus-carried transforming sequences are known to be present in all normal cells of the species of origin; corresponding, highly conserved sequences were found in foreign and sometimes quite distant species (see ref. 2). Different transforming retroviruses of the same species often carry partially or wholly different cellular sequences³, suggesting that specific cell-derived sequences could be related to specific forms of transformation³.
- Fourth, retrovirus-carried oncogenes code for transforming proteins, now well defined in several systems. The pp60<sup>src</sup> of Rous sarcoma virus (RSV)<sup>4</sup> and the p120 of the Abelson virus are among the best known examples. Although they are very

different proteins, they share an unusual protein kinase activity, leading to the preferential phosphorylation of tyrosine. It has been suggested that they may act by modifying one or more cellular polypeptides<sup>6</sup>, the identity of which is being extensively investigated. At least a portion of the pp60<sup>src</sup> and the Abelson p120 protein is associated with the cell membrane; proteins of the cytoskeleton are among the obvious candidates (for review see ref. 7).

Although intriguing, tyrosine phosphorylation cannot be regarded as a universal mechanism of transformation, as mouse cells transformed by Kirsten and Moloney sarcoma virus or SV40 virus show no increased abundance of phosphotyrosine<sup>6</sup>. Also, there is no evidence that the transforming proteins of the defective avian leukaemia viruses (DLVs) have any kinase activity. These agents have been divided into three classes8: erythroblastosis, myelocytomatosis (in vitro macrophage transforming) and myeloblastosis-type viruses. Some of these viruses have other transforming effects in addition to that indicated by their name (for details see ref. 3). The oncogenes of the three virus groups, designated erb, mac and myb, represent sequences that have been highly conserved through the phylogeny of higher vertebrates and they are unrelated to the src gene of RSV<sup>3</sup>. They are transcribed in normal cells, but at a much lower level than in transformed cells. There is a strict correlation between the presence of erb, mac and myb sequences, and the ability of the virus to transform erythroblasts, macrophage-like cells or myeloblasts. Studies using temperature-sensitive mutants<sup>9</sup> have shown that a virus-encoded protein is required to maintain the undifferentiated state of transformed erythroblasts. It has been suggested that the DLV-carried oncogenes represent normal cellular genes, coding for lineage-specific proteins involved in the control of haematopoietic differentiation. This would explain the target cell specificity of the DLVs, and suggests that the oncogene product has to be expressed continuously to maintain the differentiation block.

Recently, Hayward et al. 10 found that the slow-acting, non-defective, non-transforming avian leukosis virus (ALV) activates the cellular counterpart of the virally transmitted myc sequence, c-myc. Like other proviruses, ALV integrates randomly with the host cell genome, but occasionally it becomes inserted immediately upstream from the c-myc gene. The viral promoter, included in the long terminal repeat (LTR) sequence at the 3' end of the provirus, has been shown to be responsible for the enhanced expression of c-myc. RNA transcripts have been identified that contained both viral LTR- and c-myc-encoded information and are present at levels 30- to 100-fold higher than the corresponding c-myc RNA of the normal cell.

The 'promoter insertion model', proposed on the basis of these findings, is potentially of great interest. It suggests that a non-transforming, chronically acting virus may cause leukaemia by altering the expression of a normal cellular gene. It also raises the question of whether the activation of *c-onc* genes may

provide a common denominator between the carcinogenic action of viral and non-viral agents. I shall return to this topic in a later section dealing with the tumour-associated translocations.

The examples considered so far all suggest that overproduction of a normal cellular protein, rather than the appearance of new proteins that specifically cause transformation, is the mechanism of retroviral transformation. Is it now possible to disregard the main alternative explanation that postulates the existence of small differences between the viral oncogene product and the corresponding normal cellular product? This can only be answered from a few cases at present. The most convincing evidence comes from the joining of the normal cellular counterpart of a murine sarcoma virus oncogene (mos) with the flanking viral sequences required for infection, transcription and integration. Transfection of appropriate target cells resulted in typical transformed foci<sup>11,12</sup>, supporting the , supporting the view that retroviral transformation is not due to qualitative differences between the cellular and viral oncogene products. This conclusion was further supported by the finding that the transforming gene of a mouse sarcoma virus shows almost complete sequence homology with its cellular homologue<sup>13</sup>

If it is correct that the viral oncogene products are closely similar or identical to corresponding normal cellular proteins, but made in excessive quantities and/or in the wrong place or time, a variety of mechanisms may be explored to explain their actions. All the mechanisms start from the thesis that host-cell feedback regulations are delicately balanced and may be disrupted by relatively small changes. This may be only the first step towards fully autonomous tumours. Most naturally occurring tumours evolve by stepwise progression, and virally initiated tumours are no exception<sup>14</sup>. Autonomy, the degree of independence from growth controlling factors, is not an absolute notion—the initial transformation event may represent only a first step, and sequential modifications may lead to increased tumorigenicity.

DNA tumour viruses: These differ from the retroviruses in some fundamental respects. Whereas the retrovirally transmitted oncogenes represent an unnecessary burden as far as the viral cycle is concerned, the transforming information of the DNA tumour viruses is essential to the viral replication cycle. Moreover, while the productive cycle of the RNA tumour viruses does not interfere with cell multiplication, DNA virus replication is incompatible with cell survival, and as a consequence, the transforming information must be an early product of the viral genome. Considerable progress has been made in identifying the transforming proteins of the small papovaviruses, SV40 and polyoma.

In the SV40 system, large T antigen is the main candidate transforming protein, alone or with small T ('small t') antigen<sup>15,16</sup>. The understanding of its function is complicated by the fact that it is also involved in regulating the late viral replication cycle. Large T binds to the origin of viral DNA replication and also inhibits the synthesis of early mRNA. In microinjection experiments, a critical amount of large T antigen was required before viral DNA replication and the synthesis of virion antigens could take place<sup>17</sup>.

How can a product that must have evolved to promote the late part of the viral cycle, also be a transforming protein? One clue may be provided by the important observation of Lane and Crawford<sup>18</sup> that SV40 large T antigen is complexed to a cellular 53,000 molecular weight (53 K) protein in the nucleus of the transformed cell. A closely similar, and probably identical, 53 K protein has subsequently been found in a wide variety of transformed and tumour cells, including chemically induced mouse sarcomas, spontaneous teratocarcinomas, Abelson virus induced B-cell leukaemias, adenovirus-transformed cells and a variety (but not all) of other transformed or tumour-derived cell lines of murine and human origin<sup>19-27</sup>. Small amounts of 53 K protein were also detected in some normal mouse tissues<sup>20</sup>. A comparison of turnover rates suggests that the role of the SV40 large T antigen in transformation may relate to its ability to

stabilize 53 K<sup>20</sup>, leading to constitutively high expression. The ability of large T antigen to promote the late viral cycle would be rendered unimportant by the fact that transformation usually occurs in non-permissive cells. The ability of large T to trigger host cell DNA synthesis may also be important<sup>15</sup>.

The proposed role for the large T antigen-53 K complex in transformation received unexpected independent support from studies of an entirely unrelated transforming DNA virus system, the Epstein-Barr virus (EBV). The only virally induced product that is regularly found in EBV-transformed cells, the nuclear antigen (EBNA)<sup>28</sup>, was found to consist of a virus-specific 48 K subunit, complexed with 53 K of cellular origin<sup>29,30</sup>. A similar (probably identical) 53 K protein, not complexed to 48 K, was also present in some EBV-negative B-cell-derived lymphoma lines, but in smaller quantities than in the EBV-carrying lines. EBV conversion of these lines into stably EBNA-positive variants increased their content of 53 K protein<sup>30</sup>.

The way in which EBV results in transformation is virtually unexplored. As EBNA is always associated with transformation and is the only virally determined product known to be expressed in transformed cells, it is likely to have a key role. Recently, non-permissive cells devoid of EBV receptors have been successfully infected with EBV by microinjection<sup>31</sup> or by implantation of EBV-receptor-carrying membranes<sup>32-34</sup>. In such cells, little or no EBNA is produced and the virus enters directly into the lytic cycle. It is thus intriguing to speculate that the appearance of the EBNA-53 K complex is involved in transformation and may even prevent the cells from entering the lytic cycle. To further explore the possible role of 53 K in transformation and/or tumour progression, comparison of cell populations that have reached different stages in progression will be of considerable interest.

One such system can be provided by infecting certain EBV-negative human B-cell-derived lymphomas with EBV. This leads to a decreased serum requirement, increased resistance to saturation conditions, changed lectin agglutinability and capping behaviour and increased cloning efficiency in soft agarose 35-40, and is accompanied by a parallel increase in the amount of 53 K<sup>30</sup>. As another example, the serial propagation of SV40-transformed cell lines, an exercise in *in vitro* progression, tends to favour the selective amplification of the DNA sequences coding for large T antigen 15.41. As the amount of large T antigen and 53 K protein were found to vary in parallel 15.18.20.42, the corresponding increase in the amount of the 53 K protein may have an important selective role.

Despite their extensive functional and structural homology, the transforming functions of SV40 and polyoma virus seem to be very different. For polyoma, there is extensive evidence from both genetic and DNA transfection studies that large T is neither necessary nor sufficient for transformation<sup>43</sup>. In all probability, middle T, alone or together with small T, is responsible for the transforming function, Middle T is a 56 K protein coded by the early region of the viral genome. It shares its N-terminal region with large T but is otherwise very different, as a result of splicing of the mRNA precursor and subsequent frameshift. Like pp60<sup>src</sup>, middle T binds to the inner surface of the cell membrane and is associated with protein kinase activity<sup>43,44</sup>. There is no corresponding antigen in the SV40 system.

It has been suggested that part of the middle T sequence has originated from the host genome<sup>45</sup>, but the evidence is inconclusive. Polyoma middle T therefore still remains the most likely candidate for the role of a virally coded transforming protein. This conclusion is also supported by the fact that while polyoma-transformed cells contain the cellular 53 K protein<sup>18</sup>, it is present in an ~10 times lower concentration and is not bound to polyoma T antigen (L. Crawford, personal communication).

In conclusion, the two best known transforming DNA tumour viruses, polyoma and SV40, use very different strategies in transforming cells. For polyoma, there is a possible analogy with the transforming retroviruses insofar as transforming middle T protein has kinase activity and is associated with the inner part of the plasma membrane. For SV40, a very different mechanism,

stabilization of the transformation-related cellular 53 K protein by large T, may have a crucial role, particularly since a very similar complexing and stabilization occurs in an unrelated, but highly transforming herpesvirus, EBV.

#### Trisomy in murine leukaemia

All known murine leukaemia viruses, except the Abelson virus, are non-defective, lack direct transforming activity *in vitro*, carry no cell-derived sequences and induce leukaemia only after long latency periods. Their oncogenic effect is often dependent on prolonged viraemia and is heavily influenced by the genetics of the host<sup>46</sup>.

Most virally and non-virally induced murine T-cell leukaemias are chromosomally abnormal. Trisomy of chromosome 15 is the dominating and often the only change 47-50. Other trisomies (for example, trisomy 17) occur more rarely and usually represent an additional change to trisomy 15<sup>47,50</sup>. Trisomy 15 has also been found in B-cell lymphomas, often together with other chromosomal changes 51,52. Chromosome 15-trisomic leukaemias have been induced by Moloney virus 49,53, radiation leukaemia virus 47 (Fig. 1), X-ray radiation 4 and various chemical carcinogens 48,55,56. Most spontaneously arising AKR leukaemias were also trisomic for chromosome 15<sup>57</sup>. The cytogenetic analysis of T-cell leukaemias induced in mice carrying various types of chromosome 15 translocations has led to the conclusion that the critically important region that tends to be duplicated in the course of leukaemogenesis is localized in the distal region of the chromosome

Is the duplication of chromosome 15 a secondary consequence of leukaemogenesis, or is it directly involved in causing the transition from the preleukaemic to the leukaemic state? According to the former alternative, it could be imagined that only 15-trisomic leukaemia cells can survive among all trisomies generated by non-disjunction whereas the others are lethal. This possibility could be excluded by banding analysis of chemically (dimethylbenz(a)anthrene) and virus (Moloney) induced T-cell leukaemias, derived from three different mouse stocks, carrying different Robertsonian translocations<sup>50</sup>. The translocations were derived from the centromeric fusion of chromosome 15 with another large autosome (chromosomes 1, 5 and 6, respectively). The trisomic leukaemias contained three copies of the translocation chromosome, in most cases the only detectable chromosomal anomaly. This can only mean that the leukaemiaassociated duplication of chromosome 15 is an important event in leukaemogenesis, as it compels the centromerically fused large autosome to become a 'fellow traveller'. It also shows that the trisomy of the attached autosome is by no means lethal for the leukaemia cell.

How does trisomy 15 contribute to leukaemogenesis? Consider the AKR mouse strain, inbred and selected for high leukaemia incidence. At least four independent genetic systems are known to favour leukaemia development in this strain: (1) the integrated ecotropic (Gross-subtype) proviral DNA, localized at two sites in the genome, Akv-1 and Akv-2 (ref. 46); (2) the Fv-1<sup>n</sup> amplification system that promotes virus production<sup>59</sup>; (3) the H-2-linked Rgv-1<sup>s</sup> gene<sup>60</sup>, believed to be responsible for the deficient immune response of the AKR mouse to the leukaemia-associated antigens; and (4) a less clearly defined system that increases the likelihood of the leukaemic change directly at the level of the T cell<sup>61,62</sup>. In spite of these multiple leukaemia-favouring mechanisms, leukaemias appear only after a long latency period, in middle-aged or old mice, and are frequently trisomic for chromosome 15<sup>57</sup>.

One reason for the long latency period may lie in the requirement to generate recombinants between eco- and xeno-tropic C-type virus. Such recombinants are believed to bear a more direct responsibility for leukaemia induction than the ecotropic virus by itself<sup>63</sup>. The generation of trisomy-15 by random non-disjunction may be another time-consuming process. The promoter insertion model of Hayward *et al.*<sup>10</sup> may be considered as a third possibility. These alternatives are not



Fig. 1 G-banded karyotype and metaphase plate of leukaemia cell originating from the thymus of a male C57BL/6 mouse inoculated intrathymically with A-RadLV (radiation leukaemia virus)-induced preleukaemia cells. Note the presence of trisomy 15 (arrows).

mutually exclusive; they may represent complementary events, acting synergistically or in succession. One may envisage a broadening of the host range by recombination, leading to the reinfection of a large number of potential target cells. Random integration of the reinfecting viral genome would have no leukaemogenic consequence until the appropriate oncogene happens to be activated by the rare event of integration of the viral promoter in its neighbourhood. Duplication of the chromosome that carries the activated oncogene would help to overcome the influence of a *trans*-acting regulator, coded by the normal homologue, and experimental evidence supporting this is summarized below.

Genetic studies on the mechanisms of murine leukaemogenesis have shown that part of the genetic variation between high and low leukaemia strains acted at the level of the target T lymphocyte itself. This information came from experiments where normal thymus tissue from a high and a low leukaemia-prone strain was transplanted to the common, thymectomized F<sub>1</sub> hybrid host. Despite the fact that they were in a common host environment, exposed to the same immunological and viral influences, the thymocytes of the high leukaemia strain became leukaemic more frequently than did the cells of the low leukaemia strain<sup>61</sup>.

Analysis of trisomy 15 in leukaemias arising in  $F_1$  hybrid hosts, and related somatic hybrid studies, suggest that chromosome 15-localized gene(s) may influence the likelihood

of transformation at the target cell level. We have found  $^{55,56}$  that T-cell leukaemias induced by dimethylbenz(a) anthracene or Moloney virus in  $F_1$  hybrids derived from crosses between two mouse strains with cytogenetically distinguishable 15-chromosomes, show a highly asymmetrical trisomy. In most crosses, one of the two parental 15-chromosomes was duplicated preferentially, depending on the strain combination.

For a given genotype, the preference was the same for the virally and the chemically induced leukaemias. In crosses where the difference between the parental 15-chromosomes was only morphological but not genetic (for example, CBA×CBAT6T6 or AKR×AKR Rb6; 15) there was no significant preference, showing that the genetic content, rather than the translocated state of the chromosome, was responsible for the asymmetry. Chromosomes 15 derived from different mouse strains showed a distinct hierarchy with regard to leukaemia-associated preferential duplication. As non-disjunction of the two homologous 15-chromosomes must occur with equal frequency, this can only be interpreted to mean that the 15-chromosome-associated gene(s) that influence leukaemia development is subject to a certain variation between different mouse strains. Duplication of one of the chromosomes (in a heterozygote) is more likely to cause leukaemia than duplication of the other.

cause leukaemia than duplication of the other.

Somatic hybrid studies<sup>64</sup> have provided further evidence for the existence and probable nature of this variation. A 15trisomic AKR-derived lymphoma, designated TIKAUT, was fused with normal CBA T6T6 fibroblasts, carrying a 14; 15 translocation. Resulting highly tumorigenic segregants contained the tumour-derived chromosome 15 in five to six copies, as a rule, instead of the expected three copies. The normal parent-derived chromosome 15 was only present in one copy. Segregants of low tumorigenicity usually contained only two tumour-derived 15-chromosomes and two normal-derived 15-chromosomes. Other autosomes showed only minor random variations around the expected number, four. This suggests that the normal and the tumour-derived 15-chromosomes differ in their genetic content; the former has elements that favour and the latter that inhibit tumorigenicity in the syngeneic host. We must postulate that the duplicated chromosome 15 in the original tumour, as well as the amplified chromosome 15 in the somatic hybrid, carries a mutated form of a gene that normally controls lymphocyte growth and differentiation. Alternatively, the same gene may have been permanently activated by proviral DNA insertion. The phenotypic effects of this change would not be expressed, however, unless the chromosome is duplicated by non-disjunction, needed to overcome the suppressive influence of a *trans*-acting regulator, produced by the normal homologous chromosome. This hypothesis can be tested by cytogenetic analysis of segregants of hybrids between 15-heterozygous trisomic leukaemias and normal cells. If the hypothesis is correct, only the duplicated chromosome will be amplified in highly tumorigenic segregants.

#### **Translocations**

While the vast majority of human and animal tumours fail to show specific translocations, highly regular translocations are associated with chronic granulocytic leukaemia in humans (Ph<sub>1</sub> chromosome), with Burkitt's lymphoma and with murine plasmacytomas. This review has been limited to the latter two, B-lymphocyte-derived tumours.

Two characteristic translocations have been found in mineraloil-induced murine plasmacytomas  $^{65,66}$ . The more common marker, seen in both  $\kappa$  and  $\lambda$  producers, arises by the translocation of the distal part of chromosome 15 to chromosome 12, known to carry the heavy chain immunoglobulin locus (Fig. 2). The breakpoints are D3 in chromosome 15 and F2 in chromosome 12. The heavy chain immunoglobulin loci are located proximally to band F2<sup>67</sup>.

A reciprocal 6; 15 translocation was also seen but only in  $\kappa$  chain-producing plasmacytomas<sup>65,66</sup> (Fig. 3). Chromosome 6 is known to carry the  $\kappa$  chain locus<sup>68</sup>, although its precise location is unknown. The breakpoint on chromosome 15 was in band D3, as in the 12; 15 translocation.

In diploid or near-diploid tumours, the translocations only affected one of the two homologous chromosomes. In tetraploid plasmacytoma cells, the translocation must have occurred before polyploidization, because two of the four homologous chromosomes showed the translocation.

In each plasmacytoma clone only one of the two homologous immunoglobulin loci is responsible for the synthesis of a complete immunoglobulin molecule. It is of great interest to explore whether or not the translocation selectively affects the active or the inactive chromosome 12. This can be done by correlated cytogenetic and immunoglobulin allotype studies on plasmacytomas heterozygous for the allotypic marker and for an appropriate Robertsonian translocation.

The segment of chromosome 15 involved in the plasmacy-toma-associated translocations is localized within the region that becomes regularly duplicated in the 15-trisomic B- and T-cell leukaemias (including cases where parts of chromosome



Fig. 2 G-banded karyotype of a hypertetraploid plasmacytoma cell. Note the presence of two copies of the T(12; 15) translocation and two deleted 15 chromosomes (del 15).

Fig. 3 G-banded karyotype of a hypertetraploid plasmacytoma cell. Note the presence of two copies of the reciprocal translocation between chromosomes 6 and 15 (rcpT (6; 15)).

15 are translocated to other chromosomes)<sup>49,58</sup>. It is likely that this region carries gene(s) that control lymphocyte-plasmacyte differentiation and growth. Translocation to the immunoglobulin gene region, an area that is highly active in B lymphocytes and plasma cells, may bring the gene(s) under the influence of a highly active promoter. This could lead to the overproduction of some important regulator, in a manner analogous to retrovirally mediated oncogene transmission and the viral promoter model. It is important to explore whether the breakpoint on the immunoglobulin-locus-carrying chromosome affects the same nucleotide sequences in different tumours and how it is related to the immunoglobulin locus itself.

If studies of this type confirm that the translocation affects a specific site within or near the immunoglobulin locus, one may still ask whether this is due to some special vulnerability of this region to breakage, due to the DNA rearrangements that are known to occur in the area. This is unlikely, however, because  $\lambda$ -producer plasmocytomas showed only 12; 15 but no 6; 15 translocations<sup>69</sup>, even though the  $\kappa$  region is known to be deleted or rearranged in  $\lambda$  producers<sup>70</sup>.

The promoter activation hypothesis also implies that the translocation of the distal part of chromosome 15 to the immunoglobulin-locus-carrying chromosomes, rather than the deletion from 15, is responsible for the malignant transformation. This hypothesis can be tested by parallel chromosome segregation—tumorigenicity studies on appropriately constructed somatic hybrids.

A possibly analogous translocation has been discovered in a human B-cell-derived tumour. Both the EBV-negative and the EBV-carrying forms of Burkitt's lymphoma were found to carry a highly specific reciprocal 8; 14 translocation<sup>71-73</sup>. Banding analysis showed the involvement of the same breakpoints in different individual cases, affecting bands 8q24 and 14q32. The same translocation was also found in B-cell-derived acute lymphocytic leukaemia<sup>74,75</sup>, believed to originate from the same type of target cell as Burkitt's lymphoma. Other lymphomas were also found to carry 14q + markers in a considerable number of cases, but the variability of the breakpoints and of the donor chromosome distinguish these markers from the specifically Burkitt's lymphoma-associated 8; 14 translocation<sup>76</sup>.

Recently, variant forms of the Burkitt's lymphoma translocation have been described. In these cases, the same distal piece of chromosome 8 becomes translocated, but to chromosome 2 or 22 rather than chromosome 14<sup>77-81</sup>. The situation is reminiscent of the Philadelphia chromosome, where

a 22; 9 translocation is found in most cases, while a minority  $(\sim 10\%)$  shows a 22-deletion with the same breakpoint, but with either no identifiable translocation site or a translocation to other recipient chromosomes<sup>82</sup>.

The variant Burkitt's lymphoma translocations suggest that the distal part of chromosome 8, rather than chromosome 14, carries the gene(s) responsible for the development of Burkitt's lymphoma. As chromosome 14 is known to contain the immunoglobulin heavy chain loci in man<sup>83</sup>, the human 8; 14 translocation may be functionally analogous to the murine plasmacytoma-associated 12; 15. This concept is strongly supported by the recent demonstration<sup>84</sup> that the human  $\kappa$  gene is on chromosome 2, and the  $\lambda$  gene on chromosome 22—the chromosomes involved as recipients of the distal chromosome 8 fragment in the variant Burkitt's lymphoma translocations. Interestingly, the  $\kappa$  gene has been localized to the short arm of chromosome 2, in the same region as the breakpoint involved in the 2; 8 translocation.

#### Perspectives

Two major alternatives are now under consideration to explain the action of the virally transduced, cell-derived oncogenes. According to one hypothesis, transformation is merely a matter of gene product dosage. Due to the integration of the oncogene in the wrong place or in the wrong cell, a certain normal cellular product is synthesized in excessive quantity or at the wrong time, interfering with the normal differentiation programme. The second alternative predicts the existence of subtle qualitative differences between the virally carried oncogene and the corresponding normal cellular gene.

While the problem cannot be regarded as definitely settled, the recent successful transformation of fibroblasts with the normal cell-derived counterpart of the murine sarcoma virus-associated oncogene<sup>11,12</sup>, and even with randomly sheared DNA from normal cells<sup>85-87</sup> argues against the second hypothesis.

For tumour-associated non-random chromosomal changes such as chromosome 15 trisomy it is simplest to invoke gene dose effects. It may be objected that a change in gene dosage of 50% is a relatively minor change. On the other hand, the relationship between the normal cell and its many growth controlling signals must be extremely finely poised, so that small changes may tip the balance and favour unlimited proliferation. However, the chromosomal analysis of high and low tumorigenic segregants of somatic cell hybrids indicates that there also

exists a qualitative difference between the relevant gene(s) carried by the tumour- and the normal cell-derived chromosome 15. This is probably due to a mutation or some other change, like proviral DNA insertion. Duplication of the altered chromosome has probably overcome trans-acting regulation by the normal homologue, resulting in the leukaemic phenotype.

Both quantitative and qualitative effects must be considered in relation to the murine plasmacytoma-associated 12; 15 and 6; 15 translocations. A simple transposition of the distal region of chromosome 15, presumed to be involved in the control of lymphocyte-plasmacyte growth and differentiation, to the highly active immunoglobulin-producing gene region would be in line with the gene product dosage hypothesis, provided it can be shown that the translocation involves the active and not the allelically excluded immunoglobulin-producing chromosome and takes place within or close to the immunoglobulin gene region. Alternatively, it is possible that sequence changes ('mutations') are also involved here. Somatic hybridizationsegregation experiments involving parallel cytogenetic and tumorigenicity tests of the same type as on the trisomic lymphomas may give decisive information on this question. The Burkitt lymphoma-associated specific translocations, where the distal fragment of chromosome 8 is translocated to the heavy chain immunoglobulin-cluster-carrying chromosome 14 in typical cases or to the  $\kappa$  light chain gene-carrying chromosome 2 or the  $\lambda$  light chain gene-carrying chromosome 22 in variants, lends strong independent support to the hypothesis. Yet further support comes from the fact that the  $\kappa$  gene is localized in the short arm of chromosome 2, which is precisely where the translocation occurs (band p12).

The constitutive switch-on of cellular oncogenes is emerging as a fairly common theme in carcinogenesis. The evidence is far

from complete and there are already some examples that may not fall into this general category (transformation by some of the oncogenic DNA viruses is one of them). We must be wary of the ever-present occupational risk of the cancer researcher: generalization. With this caveat, it is quite clear that cellular oncogenes can be switched on to constitutive activity by a variety of mechanisms. In the model of direct retroviral transformation, the oncogene is switched on because it has recombined with a powerful retroviral promoter. The resulting unit can transform, no matter where it integrates. This situation is mimicked by the successful DNA-mediated transformation with large pieces of tumour-derived DNA<sup>86</sup>. In other situations, the cellular oncogene remains in place, but becomes activated due to events that occur in its immediate neighbourhood. The integration of a viral promoter is one such event, chromosomal translocation to a highly active region of the cellular genome is another. Experimentally, the occasional successful transformation by normal cell-derived small DNA fragments probably mimics the latter situation.

The identification of the oncogenes and the definition of their regulation and function in the normal cell are clearly among the most fascinating aspects of the field. Their high evolutionary conservation strongly suggests that they have essential roles in cell growth and regulation. This analysis has already erased much of the traditional conceptual and experimental barriers between chemical and viral carcinogenesis and will no doubt eventually unite them under the common umbrella of cell genetics.

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### ARTICLES

# New observational constraints on the M87 jet

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New observations at 1.6–3.45  $\mu$ m confirm the presence of a dramatic ( $\Delta \alpha \sim 1$ ) break between radio–IR wavelengths and 6,000 Å, in the spectrum of the M87 jet. These data, in combination with data taken in other spectral regions, show that the individual knots in the M87 jet have nearly the same spectral indices and nearly the same large ( $\Delta \alpha \sim 1$ ) spectral break. This large spectral break and the constancy of spectral properties between the knots pose serious constraints for models of the M87 jet.

THE M87 jet has long attracted detailed observational and theoretical interest<sup>1</sup> because of its clear relationship with non-thermal activity centred in the nuclei of galaxies. Recently this object has become the prototype for a myriad of radio galaxies which possess radio (and in some instances optical and even X-ray) jets which clearly link the galactic nucleus to the extended regions of radio emission (for a review see ref. 2).

The M87 jet is unique in that it is easily detectable over a very wide range of frequency  $(10^8-10^{17} \text{ Hz})$  and, therefore, gives us the opportunity to study the distribution of photon energies in some detail. Thus, the overall spectrum of the M87 jet may help to determine the exact manner in which energy is transported from the nucleus to the outer radio emitting regions. We now present new IR observations of the brightest knots in the M87 jet at  $H(1.6 \ \mu\text{m})$ ,  $K(2.2 \ \mu\text{m})$  and  $L(3.4 \ \mu\text{m})$ .

#### New observations

Near IR measurements of the jet of M87 were obtained on the nights of 17 and 18 February 1981, using the Steward Observatory 2.25-m telescope. The seeing on these nights was generally good and in particular was 1 arc s or better during the H measurements. Three different observing modes were used. First at  $K(2.2 \,\mu\text{m})$ , where the flux from the jet dominates the observed flux, consisted of photometry with the aperture centred on knot A, 12 arc s from the nucleus and complementary photometry centred on a patch of galaxy 12 arc s from the nucleus but 180° different in position angle. In the second mode,

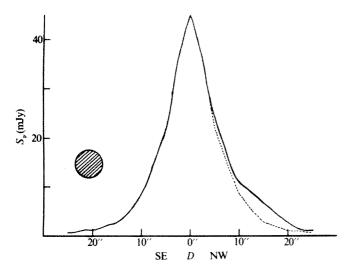


Fig. 1 Scan through the nucleus of M87 from the south-east to the north-west (jet) at  $H(1.6 \mu m)$  using a beam as indicated by the shaded circle. D is the distance from the nucleus. The dashed line on the north-west side is the reflection of the south-east (stars only) scan about the nucleus.

Table 1 IR photometry of	knots $A + B$ in the M87 jet
Wavelength (μm)	Flux (mJy)
H (1.6) K (2.2) L (3.45)	$3.8 \pm 0.2$ $5.1 \pm 0.2$ $7.2 \pm 0.6$

at  $L(3.45 \mu m)$ , where the stellar background makes only a small contribution, photometry on the jet was obtained, and the stellar contribution was estimated from the 2-µm flux of the complementary galaxy patch and assuming that K - L = 0.2. This correction amounted to only 10% of the flux observed at L. The third observing mode was at  $H(1.5 \mu m)$ , where the stellar contribution is comparable with that from the jet, and consisted of scans in the jet and anti-jet directions, registered on the nucleus. The result of this scanning at 1.6 µm is shown in Fig. 1 where the flux from the jet is clearly seen as an excess above the stellar flux. A 5.9 arc s aperture, chopping in declination, and a reference beam displaced 10 arc s in declination from the signal beam were used for all the measurements. The observations were calibrated as described by Low and Rieke3; the zero point of the magnitude scale at H was assumed to be 1,080 Jy. Data at 2.2 and 3.45 µm were taken using a liquid-nitrogen cooled InSb detector while a liquid-helium cooled InSb detector was used at 1.6 μm and to repeat the observations at 2.2 μm. In addition, the stellar contribution at K was checked by estimating the stellar flux at 1.6  $\mu$ m from the scan and assuming that H - K =0.25, the stellar colour appropriate at knot A's distance from the nucleus. This procedure yielded a stellar correction within 20% of the value observed from the complementary galaxy patch. These results are listed in Table 1.

# Comparison with observations at other wavelengths

Before these IR observations, there was still some question as to whether the radio emission from the jet arose from the same physical source as the optical emission. On the one hand recent interferometer maps of the jet by Owens et al.<sup>4</sup> and by Laing<sup>5</sup> strongly argue for a single emission mechanism as they demonstrate a one-to-one correspondence between the position, size and relative brightness of the radio and optical knots. However, there were lingering doubts about this conclusion because:

- (1) Earlier attempts to connect all the available optical, radio and IR detections and upper limits with a single smooth curve produced a violation or near violation of the  $10~\mu m$  upper limit.
- (2) The optical spectral index of 1.7 is much larger than the radio spectral index of 0.6. The procedure of fitting a smooth curve through the data required a dramatic break ( $\Delta \alpha \sim 1.0$ ) just below the lowest frequency optical observation<sup>7</sup>. Such a large

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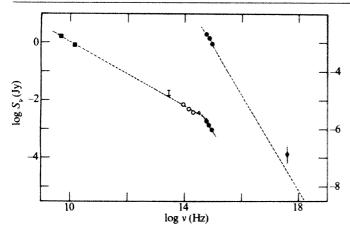


Fig. 2 The spectrum of the brightest knots (A+B) in the M87 jet. The radio ( $\blacksquare$ )<sup>4-5</sup>, IR ( $\bigcirc$ , this paper, 10- $\mu$ m upper limit from ref. 6), optical ( $\triangle$  for I (ref. 7);  $\blacksquare$ , for U, B, V (refs 6, 8, 9)), and X ray ( $\blacksquare$ ) have been normalized to a 6 arc s aperture centred on knot A, 12 arc s west and 5 arc s north of the nucleus. The X-ray point is a flux measurement of the entire jet corrected downwards by assuming a light distribution identical to that seen in the optical  $^{8,12}$ . The left-hand flux scale refers to radio-optical frequencies while the right-hand scale refers to optical-X-ray frequencies. The errors are smaller than the figure symbols except where explicitly shown.

break is twice as large as that expected due to isotropic synchroton losses and continuous particle injection.

Figure 2 shows the spectrum of the region of knots A and B(see ref. 7 for knot identification) including our IR measurements at H, K and L. All of these observations are corrected to a 6 arcs aperture at the position of knot A, 12 arcs from the nucleus. At this position the aperture includes knots A, B, I, and J. The optical observations have been corrected downward by 0.03 mag at each frequency because knot K was included in the 7-arc s aperture used  $(m_B \text{ for knot } K = 18.95 \pm 0.2)^8$ . Only if the positioning of the observing aperture were displaced 1 arcs would knot C be included, requiring an additional 0.25 mag downward correction. That this is not the case is suggested by the agreement between the photoelectric blue magnitude<sup>6</sup> and the photographically determined blue magnitudes<sup>8</sup> of knots A, B, I and J. The point at I (ref. 7) does include knot C and has been corrected downward, but it is still 0.6 mag too high to fit a smooth curve between V and H. It is not surprising that this point is the most discrepant because it is based on photographic photometry near the wavelength where the contrast between the jet and the galaxy is minimal. Note that there is no indication of recent (1964-74) optical variability in knots A and B  $(\pm 0.1 \text{ mag})^{7-9}$  although there is a claim for optical variability on a longer time scale  $(1956-78)^{10}$ .

The radio data are from ref. 5 at 15.4 GHz and ref. 4 at 5 and 15 GHz. At 15 GHZ the values of the peak flux and knot size for knots A and B from ref. 5 yield total flux values 60% higher than similar estimates from the map in ref. 4. We have used the ref. 5 value both because the larger beam size may have included flux missed by the smaller beam of the incomplete VLA and because of the uncertainty in determining peak fluxes from contour maps. From the  $805 \pm 25$  mJy value at U band<sup>5</sup>, we estimated  $1,550 \pm 60$  mJy at C band by using the spectral index between those two bands ( $\alpha = 0.59 \pm 0.08$ )<sup>4</sup>.

We have not plotted the UV data points for the jet from observations made with IUE<sup>11</sup> because there was no attempt to subtract the galaxy's contribution accurately. Although these points are very uncertain, they do suggest a continuation of the optical power law from 3,000 to 1,000 Å without a dramatic change. A recent X-ray point<sup>12</sup> is also consistent with an extrapolated power law of  $\alpha = 1.7$  to within the errors. There is also an indication that the distribution of light in X rays is similar to that in the optical. The high frequency data are thus consistent at present with a single steep power law from  $\log \nu = 14.8$  to 17.6.

The new data for the jet are consistent with a smooth spectrum with internal slopes in the radio, IR and optical of 0.6, 0.8 and 1.7. The point at I (ref. 7) is not consistent with this spectrum, but the 10 µm upper limit is no longer violated. If we demand that the extrapolation of the IR data points to higher frequencies fall above or at the point at V (ref. 6) then the IR slope must be  $0.7 \pm 0.1$ . Thus, the data now argue strongly that the radio and IR emission mechanism is the same because the measurements in both spectral regions connect up with a simple smooth curve of nearly constant slope. In fact, the present observations are still consistent with a single power law below the optical with  $\alpha = 0.7$ , although there is a slightly better fit with a gently curving spectrum steepening from 0.55 to 0.7. Either case requires a dramatic break between IR and optical and further constrains the spectrum so that a break of  $\Delta \alpha \sim 1$  most probably occurs between 5,500 and 6,500 Å and certainly within the range 4,500–8,000Å. The slope from  $H(1.6 \mu m)$  to Vis 0.6, indicating that the break cannot be far from V

The spectral break could be due to reddening, but this seems improbable because: (1) the IUE data<sup>11</sup> show no evidence for the 2,200 Å dust feature in the jet; (2) without any dereddening being applied, the V point lies slightly above the extrapolated power law from the IR—dereddening will increase this difference; (3) scans along the jet indicate spectral properties which are constant along the jet so the extinction along the jet would have to be uniform over many kiloparsecs; (4) internal reddening has not been observed in elliptical galaxies.

The scan in Fig. 1 can be used to derive the IR brightness along the jet. Figure 3 shows the results of a point-by-point subtraction in the form of a strip scan along the jet with 6 arc s resolution. The data (solid line) are compared with similar data at 15.4 GHz (ref. 5) and in the optical U filter. A similar optical scan in the B filter. is identical to within the combined 3% errors with the U filter data. We have plotted only the U filter data as its errors are slightly smaller due to the higher contrast of the jet at that frequency and to the use of a linear detector in that data set. No data are plotted inside 10 arc s from the nucleus where the galaxy subtraction causes much larger errors at optical and IR frequencies.

To within the combined errors all four scans are identical indicating extremely similar spectral indices all along the jet. Table 2 lists the radio–IR (low  $\nu$ ) and IR–U filter (high  $\nu$ ) spectral indices for the four brightest knots outside 10 arc s from the nucleus. To within quite small errors the spectra both above and below the break are identical for all four knots.

Even though no scan is available at V band, these data strongly suggest that the break frequency is identical for all four knots to within  $\pm 0.3$  in  $\log \nu$ .

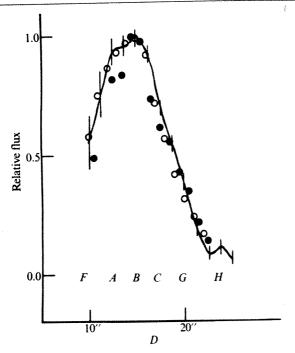
#### New constraints on jet models

These new IR observations place severe restrictions on any successful model of the M87 jet.

- (1) The optical and radio emission almost certainly arise from a single emission mechanism.
- (2) The break in the spectrum of the brightest knots is very abrupt in frequency ( $\Delta \log \nu \le 0.1$ ) and large ( $\Delta \alpha = 1$ ).
- (3) The spectra of the various knots are very similar both above and below the break frequency.
- (4) The break frequency must be nearly identical (to within 0.3 in  $\log \nu$ ) for the brightest knots.

Table 2 Spectral indices in the M87 jet						
Knot	$lpha_{ m low}$	$lpha_{ m high}$	λ <sub>break</sub> (Å)			
Α	$0.57 \pm 0.01$	$0.92 \pm 0.04$	5,500-6,500			
В	$0.58 \pm 0.01$	$0.93 \pm 0.05$	4,500-6,500			
C	$0.58 \pm 0.01$	$0.92 \pm 0.05$	4,500-6,500			
G	$0.58 \pm 0.02$	$0.88 \pm 0.09$	4,500-8,000			

 $\alpha_{\mathrm{low}} = \alpha(H~(1.6~\mu\mathrm{m}),~5~\mathrm{GHz});~\alpha_{\mathrm{high}} = \alpha(U~(0.36~\mu\mathrm{m}),~H~(1.6~\mu\mathrm{m})).$ 



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Fig. 3 Comparison of the flux distribution along the jet at three wavelengths:  $\operatorname{radio}^5(\bigcirc)$ , IR from this paper (solid line), and optical  $^{9,13}(\bigcirc)$ . The radio and optical data have been convolved with the 6 arc s beam used for the IR measurements. The error bars refer to the IR data and are due mainly to uncertainties in subtracting the stellar background in M87. At the flux peak, the errors at all frequencies are  $\sim 3\%$  and remain constant with D (distance from the nucleus) in the radio but increase towards the nucleus in the optical and IR. Beyond 20 arc s a flux of 38 mJy per beam has been subtracted from the radio points to eliminate the contribution from the extended ridge seen at 15 GHz (ref. 5). The letters refer to locations of the various knots  $^8$ .

(5) The optical and near-IR emission dominate the observed energy losses from the jet. At a distance of 15.7 Mpc (ref. 14), the luminosity of knot A alone at V is  $1.6 \times 10^{41}$  erg s<sup>-1</sup> from a region less than 25 pc across<sup>8</sup>. The electrons responsible for this radiation have  $\gamma \sim 10^6$  if we assume the equipartition value for the magnetic field strength  $(H \sim 10^{-3}~G)^{-15}$ .

In classical synchrotron theory <sup>16</sup> a high frequency spectral break may be due to either (1) a break in the electron energy distribution or (2) energy dependent losses.

In regions of low radiation flux as in extended radio sources, synchrotron emission dominates high frequency energy loses. For an isotropic particle distribution and a continuous injection of electrons the expected break is  $\Delta \alpha = 0.5$ . A similar break is expected in regions of high radiation flux where Compton scattering dominates the energy losses. A more dramatic break is possible if either: (1) the particle pitch angle distribution is highly ansotropic or (2) the injection of electrons ceased a time t yr ago where:

$$t^2 = 4 \times 10^8 (H \sin \theta)^{-3} \nu_b^{-1} \tag{1}$$

where H is the magnetic field strength in G;  $\theta$  the particle pitch angle; and  $\nu_b$  the frequency of the spectral break.

The cessation of particle injection is an attractive hypothesis because the expected  $\Delta\alpha$  matches the observations:  $\alpha_{low} = (\gamma - 1)/2 = 0.6$  to  $\alpha_{high} = (2\gamma + 1)/3 = 1.8$  (ref. 16). However, the time since cessation is extremely short compared with the light travel time between the knots or from the nucleus to the knots. For example, given an equipartition magnetic field strength of  $H \ge 10^{-3}$  G,  $t \le 30$  yr (ref. 15). The nearly identical value that we find for the break frequencies in the four brightest knots requires that the time since last particle injection into each knot is identical to within 40% of the light travel time from the nucleus to knot A, assuming a magnetic field of  $10^{-4.5}$  G. For larger fields this restriction is, of course, much worse. Therefore, an additional difficulty for the synchroton loss model for the

spectral break is that no matter how the energy is supplied (for example, electrons or protons streaming from the nucleus or in situ acceleration), and given reasonable magnetic field values  $(H \le 10^{-4.5} \, \text{G})$ , the 'shut off' time for energy input is identical from knot-to-knot to less than the light travel time from knot-to-knot. Only a favourable projection angle can save this mechanism for consideration, a possibility that could be tested by further observations of the jet in the range 4,000–8,000 Å.

The expected spectral break is also steeper in the case of a highly anisotropic particle pitch angle distribution (or distribution in H sin  $\theta$  to be more precise). For electrons at a single pitch angle an exponential cutoff is observed at high frequencies even for continuous particle injection. This is consistent with the optical and UV observations11 which approximate a very steep power law between 1,000 and 5,000Å but could exponentially decay in the unobservable EUV. However, if the observed X-ray point is an extension of the optical-UV power law, the spread in pitch angle (assuming a constant magnetic field strength) must be at least an order of magnitude in  $\sin \theta$  with more electrons populating the larger pitch angles and thus responsible for the observed optical radiation. Either of these two situations is possible if particles streaming at low pitch angles (and thus with long synchrotron lifetimes) are pitchangle-scattered when they reach the observed knots. However, the great similarity among the high frequency spectral indices of the individual knots means that the scattering process must be so uniform in knots separated by thousands of light years that the particle distributions in  $H \sin \theta$  are virtually identical from knot to knot. Perhaps knot-to-knot variations do exist in the spectra above the break but the present observations are not accurate enough to reveal them.

Based on the above, we favour interpreting the shape of the spectrum of the jet as being due to the underlying electron energy distribution. If this is true it is probably then that the X-ray flux arises from Compton scattering. Otherwise, to avoid an additional high frequency spectral break, the radiating particles should show negligible synchrotron losses even for the  $\gamma \sim 10^7$  electrons responsible for the X rays. The very short synchrotron loss time scale for these electrons (1-200 yr for  $H = 10^{-3} - 10^{-4.5}$  G) limits the size of the emitting region. An equipartition field strength of  $H \ge 10^{-3} \,\mathrm{G}$  would require that each knot be smaller than 4 m arc s. The expected Comptonscattered X-ray flux would then exceed the observed flux by about an order of magnitude<sup>17</sup>. This discrepancy can be reduced but not entirely removed by assuming a much smaller magnetic field, or by requiring the acceleration to take place along a line or in a very thin slab as might be expected with a shock front acceleration model. Thus, the X-ray radiation can be of synchrotron origin only if anisotropies in the radiation field and the electron velocity distribution reduce the Compton scattering substantially.

The inverse Compton origin of the X-ray radiation can be tested by observing the overall jet spectrum in the Einstein band. A second frequency observation would be expected to detect the difference between the expected slopes for a synchrotron ( $\alpha = 1.7$ ) and inverse Compton ( $\alpha \le 0.8$ ) origin for the X rays. The inverse Compton X-ray slope is expected to be the same as the observed low frequency synchrotron slope ( $\nu \le 10^{14}$ ).

If the photon spectrum does reflect the underlying electron particle distribution, there are two possibilities for the origin of these electrons:

First, in situ acceleration overcomes the difficulties due to synchrotron losses described above but whatever acceleration process is responsible for the high energy electrons, it must be remarkably uniform in sites separated by thousands of light years in M87. As in the particle pitch angle scattering model for the high frequency break, the expectation for in situ acceleration models is that the different knots will have different spectra reflecting variations in acceleration parameters (for example, magnetic field strength and length scale over which the acceleration process takes place).

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The large characteristic  $\gamma$  s of  $10^6$  in the jet also pose a problem for statistical acceleration mechanisms involving magnetic fields. Typically  $\gamma \sim H^2 dr/c$  for these types of processes (for example, Fermi or betatron acceleration where  $H^2/c$ is the magnetic field energy and dr is the length scale over which it is applied). Thus, for an equipartition field and knot sizes between 2 and 20 pc, the expected  $\gamma$  s are only  $10^{2-3}$ . However, large ys are expected for processes whereby electrons gain energy due to motion through an electric field such as could be produced at a shock front moving through a region containing a magnetic field<sup>18</sup>. For this class of models  $\gamma \sim qH \, dr/c$  (q is the electron charge) and thus gives characteristic  $\gamma$ s of  $10^{6-7}$  for the above parameters. This electric field acceleration model is also much less sensitive to variations in source size as  $\gamma(r) \propto r^{-1}$ instead of  $r^{-3}$  for the magnetic acceleration models. However, in either case knot-to-knot variations would still be expected and have not yet clearly been seen.

Second, an external source of particle energy is possible if a proton beam is emanating from the nucleus1. This removes the problem of synchrotron losses and naturally explains the identical spectra of all the knots. Such a proton beam produces electrons by p-p collisions and subsequent muon decay into energetic electrons and  $\gamma$  rays. Using the formalism of Gould and Burbidge<sup>19</sup>, Felten<sup>1</sup>, derives the relationship between the  $\gamma$ -ray flux  $\phi(>E)$  (photons cm<sup>-2</sup> s<sup>-1</sup>) above an energy E (eV) and the observed synchrotron spectrum  $F(\nu_e)$  in W m<sup>-2</sup> Hz<sup>-1</sup> with slope s where  $\nu_e = 10^{-6}$  HE<sup>2</sup>:

$$\phi(>E) = 7 \times 10^{8} [a(2s+1)]^{-1} (0.2)^{s} HEF(\nu_{e})$$
 (2)

where H is the magnetic field in G and a(s) is a function of spectral index s (see ref. 20, p. 68). As the dominant energy electrons occur at the break frequency  $\log \nu_e = 14.75$ , for an equipartition field of  $H = 10^{-3}$  G the  $\gamma$ -ray energies associated with break frequency photons are  $E = 8 \times 10^{11}$  eV. These are  $\gamma$ s in the range to which Cerenkov light detectors are sensitive. Equation (2) predicts  $\gamma$ -ray fluxes of  $10^{-11}$  photons cm<sup>-2</sup> s<sup>-1</sup>, an order of magnitude less than the present upper limit on the  $\gamma$ -ray flux from M87 above 10<sup>11</sup> eV (ref. 21).

If we assume that the magnetic field strength is the equipartition value, then there is a relationship between the observed

angular size of the knots and the  $\gamma$ -ray for the M87 jet in the proton beam model:

$$\phi(>10^{11} \text{ eV}) \approx 1 \times 10^{19} \theta^{-6/7} \text{ d} \exp(-28.8 + 0.55 \log \theta)$$
 (3)

where  $\theta$  is the angular size in m arc s. Unfortunately equation (3) predicts only an order of magnitude increase in  $\phi$  between  $\theta = 300$  and 1 m arc s. The  $\gamma$ -ray flux decreases almost as fast as the magnetic field strength decreases away from equipartition; therefore, the proton beam mechanism is easily consistent with present observation.

We conclude that there are four observations which will constrain theoretical models of the M87 jet even more than the present observations:

- (1) Flux measurements for each knot in the jet between 4,000 and 8,000 Å to determine more accurately the high frequency spectral index and break frequency for the knots. These observations are crucial in determining whether an in situ process (acceleration or pitch angle scattering) is operating in the jet. These observations require a two-dimensional linear detector to subtract the contribution from the galaxy, which is quite strong at these wavelengths.
- (2) Speckle interferometry and VLBI observations of the knots to determine more accurately the angular size of the knots and thus the equipartition magnetic field strength and inverse Compton fluxes. The optical size is crucial because of longer synchrotron lifetimes, the knot sizes may be larger in the radio than in the optical because of particle diffusion away from the acceleration region.
- (3) Improved  $\gamma$ -ray observations at high energies, to constrain the proton beam model.
- (4) A second frequency X-ray observation at HRI resolution (≤3 arc s) to determine the X-ray spectral index and thus whether the X-ray radiation is due to a high energy tail of the optical synchrotron emission or to inverse Compton scattering from lower energy ( $\nu \le 10^{14} \text{ Hz}$ ) photons.

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# Sm-Nd age of Kambalda and Kanowna greenstones and heterogeneity in the Archaean mantle

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A precise Sm-Nd age of 2,790  $\pm$  30 Myr and an initial ratio corresponding to  $\varepsilon_{Nd} = +3.4 \pm 0.6$  has been determined for the Kambalda sequence from the eastern Yilgarn Block of Western Australia. The high initial 143Nd/144Nd implies a long-lived light rare-earth element-depleted (high Sm/Nd) source for the Kambalda volcanics and therefore provides definitive evidence for ancient heterogeneities in the Archaean mantle.

THE Sm-Nd isotopic systematics have been examined in maficultramafic and felsic volcanics at Kambalda and Kanowna in the Yilgarn Block of Western Australia. This study aims (1) to determine the original ages of the volcanic complexes, which have been difficult to establish by other methods, and (2) to determine their initial <sup>143</sup>Nd/<sup>144</sup>Nd as a means of further understanding the evolutionary history of the crust and mantle. The Kambalda and Kanowna volcanics are typical of the greenstone belts which occur within the gneissic and granitic rocks of the Eastern Goldfields Province and have similarities with Archaean greenstones from other cratons. For example, the common occurrence of spinifex-textured Mg-rich basalts and peridotites of komatiitic affinity are analogous to those found in the Barberton area of southern Africa and Munro Township, Canada<sup>1-4</sup>.

Despite intensive isotopic studies, the original ages of extrusion for the greenstones in this part of the East Yilgarn Block remain equivocal. Rb-Sr and Pb-Pb age determinations<sup>5-8</sup> register specific events within the range ~2,760-2,400 Myr. However, because of the extensive metamorphic and metasomatic history of the terrain, even the oldest of these is interpreted as a minimum for the original igneous ages. Recent applications of the Sm-Nd method to dating of greenstones from other Archaean terrains<sup>9-12</sup> have shown rare earth elements (REE) are much less mobile during metamorphism, so that the Sm-Nd system may be more conclusive for the definition of original ages. Due to the lack of preservation of primary mineralogy, the present study is based on whole rock analyses.

Volcanics have been analysed from two localities (see Fig. 1), Kambalda which is composed mainly of mafic and ultramafic extrusives and Kanowna where felsic volcanism predominates. Tentative stratigraphic correlations <sup>13,14</sup> suggest that Kanowna is older, being part of a first volcanic cycle while Kambalda is thought to be at the base of a second volcanic cycle.

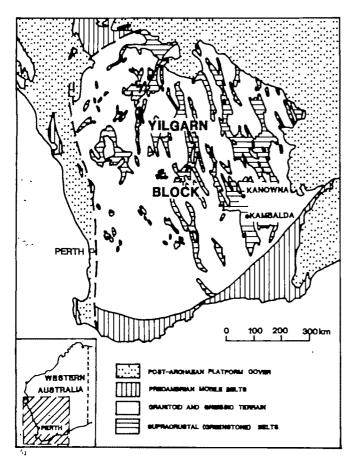


Fig. 1 Geological map of the Yilgarn Block of Western Australia showing locations of the Kambalda and Kanowna greenstone sequences.

Table 1 Sm-Nd isotopue data								
Sample		Sm (p. <sub>1</sub>	Nd pm.)	147Sm/144Nd*	143Nd/144Nd†			
Kambalda								
Footwall	72-5	1 83	5 51	0.2005	$0.512094 \pm 24$			
	72-13	1.54	4 80	0 1941	$0.511948 \pm 30$			
	72-19	2 10	6.52	0 1948	$0.511954 \pm 20$			
Hanging wall	72 <del>-9</del> 16	1.97	6.60	0.1809	$0.511716 \pm 18$			
	72- <del>9</del> 10	1.78	6.04	0.1783	$0.511680 \pm 32$			
Ultramafic	KR3	0 79	2,14	0.2234	$0.512537 \pm 24$			
	72–42b	1 03	5 20	0.1198	$0.510449 \pm 18$			
		1 03	5.22	0.1197	$0.510409 \pm 26$			
Na-granite	71–751	1 91	12.55	0.0920	$0.510088 \pm 20$			
	71-752	2 16	13.15	0.0995	$0.510226 \pm 18$			
Felsic porphyry	<b>72–4</b> 1	2.11	13.52	0.0946	$0.510131 \pm 32$			
Kanowna								
Basalt	80-184	2.78	11 39	0.1477	$0.511211 \pm 18$			
	80-185	2,46	10 04	0 1481	$0.511122 \pm 22$			
Ultramafic	80-186	0 41	1.32	0.1896	$0.512065 \pm 20$			
Felsic porphyry	80-187a	3.12	18.49	0.1019	$0.510280 \pm 26$			
	80–187b	4.23	25.59	0.0999	$0.510247 \pm 18$			
Decite Tuff	80–188	<b>4.</b> 10	23.81	0.1043	$0.510196 \pm 18$			

<sup>\*</sup> Uncertainties ±0.1%. Tracer solutions of <sup>150</sup>Nd and <sup>147</sup>Sm were calibrated using mixed normal solutions prepared from ultrapure pieces of Nd and Sm metal (Ames Laboratory) as gravimetric standards.

† Measured on spiked samples; normalized to  $^{144}$ Nd/ $^{142}$ Nd = 0 636151. The value for BCR-1 determined in this laboratory is 0 51184 ± 2.

The Kambalda volcanics form a conformable succession consisting of an ultramafic unit some 240-600 m thick sandwiched between two basaltic sequences15. The lower basalt (the 'footwall basalt') is dominantly a massive metatholeiite with pillowed and flow-top breecia horizons and has many geochemical affinities with modern-day oceanic tholeiites4. The upper 'hanging wall' basalt is more heterogeneous and contains a lower variolitic-textured section separated by a sulphide rich sedimentary horizon from an upper massive fine-grained metatholeiite. The Mg-rich ultramafic sequence consists of a large number of flow units many of which have spinifex textures. Major nickel sulphide deposits occur at the base of the ultramafic sequence. Intruding the mafic-ultramafic sequence is a series of felsic intrusives of which a large portion consists of a 'sodic-granite' 15. It has been suggested that these intrusives are recrystallized porphyries representing the subvolcanic expression of felsic volcanism within the mafic-ultramafic succession. The Kanowna area consists of a major felsic volcanic complex which overlies a sequence of metabasalts and ultramafics13. The metabasalts consist of a sequence of pillowed, amygdaloidal basaltic flows within which occurs several metaperidotites. These rocks are thought to be the stratigraphically lowest unit in the Kalgoorlie area. The overlying felsic volcanics consist of lapilli tuffs, pyroclastic flows, agglomerates and a conglomerate containing felsic and ultramafic clasts in a predominately felsic volcanogenic matrix. Intruding this sequence is an altered quartz-rich porphyry. Gold has been produced from a quartz reef in the conglomerate.

#### Results

The procedures used for the isotopic analyses of Sm and Nd are described elsewhere <sup>17</sup>. In this study, samples were totally spiked and dissolved using HF, HClO<sub>4</sub> and HCl. Measurements were made of Nd<sup>+</sup> using triple filaments. The total Nd blank is 100 pg and is therefore negligible for our study. The  $\varepsilon_{\rm Nd}$  values are defined as:

$$\varepsilon_{Nd} = \left[ \frac{\binom{143}{Nd} \binom{144}{144} Nd)_{maxt}^{T}}{\binom{143}{Nd} \binom{144}{144} Nd)_{CHUR}^{T}} - 1 \right] \times 10^{4}$$

where  $(^{143}\text{Nd})^{144}\text{Nd})^{T}_{\text{CHUR}} = (^{143}\text{Nd})^{144}\text{Nd})^{0}_{\text{CHUR}} - (^{147}\text{Sm})^{144}\text{Nd})^{0}_{\text{CHUR}} = (^{147}\text{Sm})^{144}\text{Nd})^{0}_{\text{CHUR}} = (^{147}\text{Sm})^{144}\text{Nd})^{0}_{\text{CHUR}} = (^{147}\text{Sm})^{144}\text{Nd})^{144}_{\text{CHUR}} = 0.511836$  and  $(^{147}\text{Sm})^{144}\text{Nd})^{0}_{\text{CHUR}} = 0.1967$ ;  $\lambda_{\text{Sca}} = 6.54 \times 10^{-12} \text{ yr}^{-1}$ .  $(^{143}\text{Nd})^{144}\text{Nd})^{144}_{\text{min}}$  is the measured ratio in the rock, corrected for decay since the time of

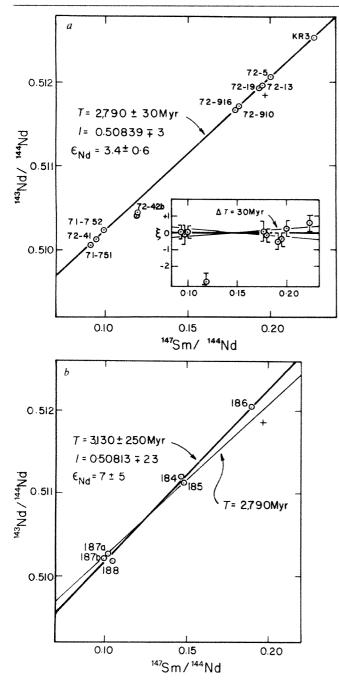


Fig. 2 a, Sm-Nd evolution diagram for the Kambalda volcanic sequence. The precise age and initial  $^{143}$ Nd/ $^{144}$ Nd result from combining the basalts and ultramafics with an associated sodic granite and felsic porphyry. A less precise age of  $2.910\pm170$  Myr is obtained from the footwall, hanging wall and ultramafics volcanics alone. +, The present-day chondritic value  $^{26}$ . b, Sm-Nd evolution diagram for the Kanowna volcanics. The large deviations from a best fit line do not allow a precise age determination.

crystallization T. The  $^{147}$ Sm/ $^{144}$ Nd enrichment factor relative to 'CHUR' is given by

$$f^{\text{Sm/Nd}} = \left[ \frac{(^{147}\text{Sm}/^{144}\text{Nd})_{\text{meas.}}}{(^{147}\text{Sm}/^{144}\text{Nd})_{\text{CHUR}}^0} - 1 \right]$$

The results of the isotopic measurements are listed in Table 1 and shown in Fig. 2a, b. Figure 2a shows the whole rock results from Kambalda. The footwall, hanging wall and ultramafic sequences from Kambalda have a relatively restricted range in Sm/Nd of  $\sim \pm 10\%$  and approximately chondritic relative abundances of REE (Fig. 3). In contrast, the sodic-granite and associated porphyries have pronounced light rare-earth element (LREE) enrichments (low Sm/Nd). Combining all the

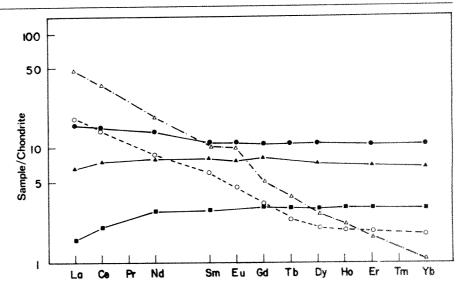
Kambalda samples yields a large range of Sm/Nd and therefore a large range in  $^{143}\text{Nd}/^{144}\text{Nd}$  of 48  $\epsilon_{\,\text{Nd}}^{\,0}$  units. As shown inset in Fig. 2a, all the data points apart from an altered ultramafic lie on the best fit line within analytical uncertainty, and define an age of  $2,790 \pm 30$  Myr  $(2\sigma)$ , an initial  $^{143}$ Nd/ $^{144}$ Nd =  $0.50839 \pm 3$   $(2\sigma)$ . If it is assumed that the sodic-granite and felsic porphyry are isochronous with and have the same initial <sup>143</sup>Nd/<sup>144</sup>Nd as the footwall, hanging wall and ultramafic volcanics, then the Sm-Nd age may represent the time of crystallization of this complex. This joint age is the same within errors as previous Pb-Pb (ref. 8) age determination for the sodic-granite of  $2.760 \pm 70$  Myr. The strict co-linearity of the felsic and ultramafic data supports this contention. A less precise age of 2,910 ± 170 Myr is obtained from the footwall, hanging wall and ultramafic volcanics alone. The lower precision of  $\pm 170$  Myr is due to their smaller range in Sm/Nd. An ultramatic from the alteration zone at the felsic porphyry-ultramafic contact is the only discrepant sample. One explanation is that the Sm-Nd isotopic system in this sample has been disturbed by contamination with non-radiogenic Nd during a late alteration process associated with the emplacement of the felsic porphyry. The unusual LREE enrichment and abundances in the ultramafic (Fig. 3) is consistent with this interpretation. Alternatively the age obtained by combining the footwall, hanging wall and ultramafic volcanics with the altered ultramafic  $(3,100 \pm 100 \text{ Myr})$  could be registering a time of early alteration. However, this is unlikely due to the similarity between the LREE enrichments observed in the altered ultramafic and the younger felsic porphyry. The initial  $^{143}$ Nd/ $^{144}$ Nd obtained from the  $2,790\pm30$  Myr isochron lies above the CHUR evolution curve ( $\varepsilon_{Nd} = 3.4 \pm 0.6$ ).

Figure 2b shows the results from Kanowna. The samples show similar variation of Sm/Nd with bulk composition, the ultramafic having the highest Sm/Nd, metabasalts and intermediate Sm/Nd and the felsic samples the lowest Sm/Nd. Despite a range in  $^{143}$ Nd/ $^{144}$ Nd of 36  $\varepsilon_{\rm Nd}^0$  units, the Kanowna samples produce a very imprecise age of  $3,130 \pm 250$  Myr and initial  $^{143}$ Nd/ $^{144}$ Nd of  $0.50813 \pm 23$  ( $\varepsilon_{Nd} = 7 \pm 5$ ). For comparison, the Kambalda isochron is also shown in Fig. 2b. The large deviations from the best fit line of the Kanowna data are in distinct contrast with the Kambalda data and could be due to significant differences in age, initial <sup>143</sup>Nd/<sup>144</sup>Nd or redistribution of Sm and Nd during later metamorphisms. In contrast to the Kambalda samples which are from deep drill-cores, the Kanowna samples are from the surface outcrops so we cannot exclude the possibility that their Sm-Nd isotopic system has been disturbed by recent weathering. As far as can be ascertained, there is no obvious difference in the extent of modification of equivalent rock types at Kanowna and Kambalda. For example, metabasalts from Kanowna and Kambalda have similar major element chemical contents (for example K<sub>2</sub>O < 0.1%) and ocelli bearing samples have been analysed<sup>7</sup> from both localities (80–184 and 72–910, 72–916). Although disturbance of the Sm-Nd system by later metamorphic and metasomatic events cannot be discounted (for example, sample 72-42b) the consequences of age and initial <sup>143</sup>Nd/<sup>144</sup>Nd variations will now be considered.

#### Sm-Nd whole rock isochrons

The evolution of initial <sup>143</sup>Nd/<sup>144</sup>Nd with time in terrestrial rocks of varying ages has been shown <sup>9-12,18,24-26</sup> to approximate the evolution expected in a reservoir having a fixed, chondritic Sm/Nd. For this reason, rocks which are closed systems and derived from the reference mantle reservoir (CHUR) will produce isochrons that pivot about the present-day values of Sm/Nd and <sup>143</sup>Nd/<sup>144</sup>Nd in CHUR. This is shown in Fig. 4 for isochrons of age 1,000, 2,000 and 3,000 Myr. For example, a suite of rocks derived from a chondritic reservoir 3,000 Myr ago will today produce a linear array with the <sup>143</sup>Nd/<sup>144</sup>Nd being proportional to Sm/Nd. A sample that has the same Sm/Nd as in the chondritic reservoir will continue to evolve in a manner identical to the reference reservoir. This applies to rocks of all ages and thus the present-day values of Sm/Nd and <sup>143</sup>Nd/<sup>144</sup>Nd

Fig. 3 Chondrite normalized REE abundance patterns for the Kambalda volcanics<sup>27,33</sup>, sodic-granite<sup>28</sup> and altered ultramafic. The unusual LREE enrichment of the altered ultramafic may be due to contamination by LREE from the younger sodic-granite. △, Na-granite; ♠, hanging wall basalt; ○, altered ultramafic; ♠, footwall basalt; ■, ultramafic.



in CHUR will be a fixed point common to all isochrons derived from CHUR. Thus samples with Sm/Nd approximately the same as in CHUR ( $f^{\text{Sm/Nd}} \approx 0$ ) will not provide meaningful age information. Samples which are most sensitive to age variations are those with non-chondritic Sm/Nd ( $f^{\text{Sm/Nd}} \neq 0$ ). These systematics have important implications for this study (and others) as it has been necessary to combine felsic volcanics ( $f^{\text{Sm/Nd}} \sim -0.5$ ) with mafic and ultramafic volcanics ( $f^{\text{Sm/Nd}} \sim 0$ ) to obtain an adequate range in Sm/Nd for precise dating. At Kambalda the sodic granite and felsic porphyry (the low Sm/Nd end-members) intrude the mafic-ultramafic sequence and therefore the  $2.790\pm30$  Myr 'isochron' represents only a minimum age for the mafic-ultramafic volcanics. It is possible, within the analytical uncertainties, that a significant age difference of  $120\pm200$  Myr may exist between the mafic-ultramafic volcanics and felsic intrusives.

The disparate Kanowna data clearly imply disturbance of the Sm/Nd isotopic system or age and initial <sup>143</sup>Nd/<sup>144</sup>Nd variations. For example, the large deviations between the low Sm/Nd felsic rocks requires that the felsic porphyry 187 has either a higher initial <sup>143</sup>Nd/<sup>144</sup>Nd or is younger than the dacite 188. The geological field relationships are consistent with the latter explanation. The basalts 184 and 185 are spatially closely related suggesting that their deviations are more probably due to variable initial <sup>143</sup>Nd/<sup>144</sup>Nd. The contrast between the Kambalda and Kanowna data may be due to dominance of felsic volcanism at Kanowna. Felsic volcanics are chemically more evolved, implying isotopically more evolved and heterogeneous mantle or crustal sources.

The Sm-Nd model age  $^{18,19}$   $(T_{CHUR}^{Nd})$  is the time in the past when the major REE fractionation in a rock occurred during its derivation from the model mantle reservoir CHUR. In Figs 2 and 4 this is equivalent to the age defined by the line joining the sample with the present day values of 143Nd/144Nd and Sm/Nd in the chondritic reservoir CHUR. Thus only fractionated (nonchondritic Sm/Nd) samples define precise model ages. The T<sub>CHUR</sub> model ages from the low Sm/Nd Kambalda felsic volcanics, 2,510-2,540 Myr, are the same within analytical uncertainty whereas the Kanowna felsic volcanics have a range in  $T_{CHUR}^{Nd}$  of 2,490-2,690 Myr. All these model ages are significantly younger than the ages indicated by the whole-rock isochrons. This discrepancy is due to the higher initial <sup>143</sup>Nd/<sup>144</sup>Nd of the whole-rock isochrons compared with that in CHUR. This can be seen in Fig. 2a, b where the best fit lines to the mafic-ultramafic samples have a higher <sup>143</sup>Nd/<sup>144</sup>Nd relative to CHUR for a chondritic Sm/Nd. The higher initial  $^{143}$ Nd/ $^{144}$ Nd relative to CHUR (corresponding to  $\varepsilon_{Nd} = 3.4 \pm$ 0.6 for Kambalda and  $\varepsilon_{Nd} = 7 \pm 5$  for Kanowna) is essentially independent of age for the mafic-ultramafic samples. The possible explanations for the well defined Kambalda point are discussed below.

#### Heterogeneous Archaean mantle

The well-defined initial 143Nd/144Nd of the Kambalda volcanics corresponding to  $\varepsilon_{Nd} = 3.4 \pm 0.6$  provides some of the first definitive evidence for ancient long-lived heterogeneities in the Archaean mantle. This can be seen in Fig. 5a where the initial <sup>143</sup>Nd/<sup>144</sup>Nd determined on Archaean igneous rocks are summarized on a graph of  $\epsilon_{Nd}$  versus time. Most of the previously determined 2,600-2,800 Myr data points cluster near the  $\varepsilon_{Nd}(T) = 0$  line suggesting that the Archaean mantle was relatively undifferentiated and the Sm/Nd of the Earth was uniform (within about  $\pm 3\%$ ) and close to the average chondritic value used to calculate the CHUR curve26. The Kambalda point (solid) lies distinctly above the CHUR curve and unambiguously implies a mantle source with  $f^{\text{Sm/Nd}} > 0$  for time scales > 100Myr. Its position above the CHUR curve is significant as the interpretation of data which lies below the CHUR curve is complicated by the possibility that the magmas may be contaminated with pre-existing continental crustal rocks with negative  $\varepsilon_{Nd}(T)$  values. This is exemplified by the Stillwater point<sup>20</sup>. Evidence for the derivation of magmas from positive  $\varepsilon_{Nd}$  mantle has been presented for Archaean gneisses from eastern India by Basu et al.31 and for Proterozoic rocks by DePaolo34

The Sm/Nd enrichment factor  $(f^{\text{Sm/Nd}})$  of the Kambalda magma source can be calculated as a function of the time (Ts)

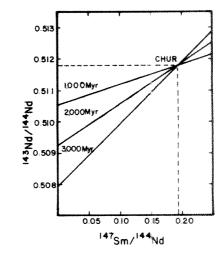
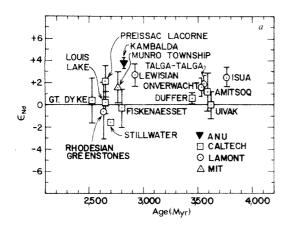


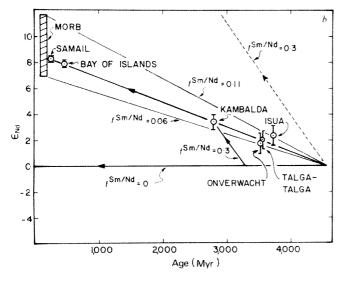
Fig. 4 Sm-Nd evolution diagram for isochrons of age 1,000, 2,000 and 3,000 Myr. Due to the approximate chondritic Sm/Nd evolution of the mantle 9-12,18,24-26, mantle-derived samples with Sm/Nd approximately the same as chondrites (CHUR) do not alone provide age information.

that it became fractionated relative to CHUR by using the relationship 18:

$$\varepsilon_{\rm Nd}(T) = f^{\rm Sm/Nd}Q_{\rm Nd}(Ts-T)$$

For Ts equal to the age of the Earth (Ts = 4,550 Myr, T = 2,790 Myr,  $Q_{Nd} = 0.0251 \text{ Myr}^{-1}$ ),  $f^{\text{Sm/Nd}} = 0.08$ . This is a minimum value and implies a mantle source with a time average Sm/Nd from 4,550 Myr to 2,790 Myr that was 8% higher than CHUR. This evolution curve is shown in Fig. 5b together with the initial <sup>143</sup>Nd/<sup>144</sup>Nd of Kambalda and the older mafic-ultramafic sequences, Talga-Talga<sup>32</sup>, Onverwacht<sup>11</sup> and Isua<sup>10</sup>. The colinearity of these data suggest the possible existence of an ancient (>4,000 Myr) ultramafic mantle source with f0.08. This reservoir would today have  $\varepsilon_{Nd} \sim +9$  and could thus be the source of the positive  $\varepsilon_{Nd}$  oceanic basalts. This is contrary to previous suggestions<sup>29,30</sup> of a younger (1,800-2,000 Myr) source for modern day oceanic basalts. To distinguish between these evolutionary models it is apparent that additional high precision initial <sup>143</sup>Nd/<sup>144</sup>Nd are required from Precambrian oceanic basalts of different ages.





**Fig. 5** a, Fractional deviations in parts per  $10^4$  of initial  $^{143}$ Nd/ $^{144}$ Nd of Archaean rocks $^{9-12,18-20,23-26,32}$ , from evolution in the CHUR reservoir  $^{18}$ . The  $\varepsilon_{\rm Nd}$  value of  $+3.4\pm0.6$  for the Kambalda sequence implies derivation from a long-lived LREE depleted (high Sm/Nd) source. b, Possible evolution of <sup>143</sup>Nd/<sup>144</sup>Nd based on the initial <sup>143</sup>Nd/<sup>144</sup>Nd of Kambalda, Isua<sup>10</sup>, Onverwacht<sup>11</sup> and Talga–Talga<sup>32</sup>. The single evolution curves with f<sup>Sm/Nd</sup> of from 0.06 to 0.10 can account for the initial <sup>143</sup>Nd/<sup>144</sup>Nd of these samples as well as present day oceanic basalts. An alternate explanation for the high initial <sup>143</sup>Nd/<sup>144</sup>Nd of the Kambalda sequence involves multi-stage evolutions with  $f^{\text{Sm/Nd}} \leq 0$ . For comparison initial  $^{143}\text{Nd}/^{144}\text{Nd}$  and inferred evolution of some highly fractionated ( $f^{\text{Sm/Nd}} \simeq 0.3$ ) Apollo 12 and 17 lunar maria basalt sources  $^{21,22}$  are also shown (dashed line).

An ancient (>4,000 Myr) high f<sup>Sm/Nd</sup> mantle source would imply an early differentiation of the Earth as well as incomplete mantle mixing to allow the preservation of these distinctive magma reservoirs. We note that this hypothesis is independent of the exact CHUR evolution curve for the bulk Earth but may be influenced by possible interlaboratory biases between the Kambalda and Onverwacht, Talga-Talga, Isua data. The initial <sup>143</sup>Nd/<sup>144</sup>Nd of the Apollo 12 and 17 lunar maria basalts <sup>21,22</sup> is also consistent with an early differentiation (~4,500 Myr) of planetary bodies resulting in the formation of even more highly fractionated magma reservoirs having  $f^{\text{Sm/Nd}} = 0.3$ .

An alternative interpretation of the Kambalda initial ratio is that the time of differentiation of the source is substantially younger than 4,500 Myr. This would require a more strongly LREE depleted source. For example, for  $T_s = 3250 \text{ Myr}$ ,  $f^{\text{Sm/Nd}} = 0.3$ , which by analogy with the lunar data, is probably a maximum  $f^{\text{Sm/Nd}}$  value.

#### **Conclusions**

The Sm-Nd whole rock systems have been examined in two volcanic sequences from the eastern Yilgarn Block of Western Australia. The predominantly mafic-ultramafic sequence at Kambalda has preserved uniform and regular Sm-Nd systems consistent with a well defined crystallization age of  $2,790 \pm 30$ Myr and an initial  $^{143}$ Nd/ $^{144}$ Nd = 0.50839 ± 3. In contrast, data from the felsic Kanowna complex are highly dispersed with a poorly defined age of  $3{,}130 \pm 250$  Myr and initial  $^{143}$ Nd/ $^{144}$ Nd =  $0.50813 \pm 23$ . The differences between the two sequences are poorly understood but may reflect disturbance of the Sm-Nd system or large variations in the age and initial ratios of the Kanowna complex. Due to the deviant Kanowna data array, the age difference between Kanowna and Kambalda is not considered to be significant although it is consistent with inferred stratigraphic correlations 13,14

Although the whole rock samples from Kambalda form a perfect linear array (apart from an altered ultramafic) the age and initial <sup>143</sup>Nd/<sup>144</sup>Nd is constrained by combining maficultramafic volcanics with felsic volcanics. Consequently any age differences which may exist between felsic and mafic volcanics are not readily apparent. For example, a plausible hypothesis for the formation of the Kambalda complex may be the extrusion of the mafic-ultramafic volcanics in an ocean basin at  $2,910 \pm 170$ Myr (age given by the mafic and ultramafic volcanics only). Then, within  $120 \pm 200$  Myr (that is at 2,790 Myr) the sodic granite and related felsic intrusives were produced by partial melting of an eclogite source. An eclogite or more specificially a garnet bearing source<sup>28</sup> is implied by their pronounced LREE enrichments ( $f^{\text{Sm/Nd}} \ll 0$ ) and may be the subducted equivalents of the mafic and ultramafic volcanics now preserved in the Kambalda complex.

Regardless of the question of the original age, the initial <sup>143</sup>Nd/<sup>144</sup>Nd of the Kambalda sequence is significantly greater than the chondritic evolution curve with an  $\varepsilon_{Nd}$  value of  $\pm 3.4 \pm$ 0.6. For the sodic-granite alone, the  $\varepsilon_{Nd}$  value is independently set at +3.9, if the Pb-Pb<sup>8</sup> age of 2,760 Myr is used. The high initial <sup>143</sup>Nd/<sup>144</sup>Nd requires an earlier LREE depleted (high Sm/Nd) source for the Kambalda volcanics and therefore provides some of the first definitive evidence for long-lived heterogeneites in the Archaean mantle.

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# Structure of vitamin D-dependent calciumbinding protein from bovine intestine

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The structure of vitamin D-dependent calcium-binding protein from bovine intestine, determined crystallographically to 2.3 Å, contains a pair of calcium-binding domains similar to those in parvalbumin, but one domain has a longer and rearranged calcium-binding loop. This result has implications for structure predictions of other calcium-binding proteins such as calmodulin and S-100.

THE role of calcium as a 'second messenger' in the regulation of intracellular processes is now well established.1,2, and requires that intracellular calcium concentrations be precisely controlled. A wide variety of calcium-binding proteins, with affinity constants<sup>3</sup> in the range 10<sup>-8</sup>-10<sup>-5</sup> M, have been identified which either participate in this control or exhibit activities modulated by calcium (ref. 4 and refs therein). One such class of proteins comprises the vitamin D-dependent calcium-binding proteins, which have been studied in various species. In birds, a protein of molecular weight (MW) of ~28,000 is found in the intestine, kidney, brain, shell gland and other organs. In mammals, a protein of similar size is found in various tissues, but the predominant intestinal calcium-binding protein is a smaller, 10,000-MW species<sup>5</sup>. The chicken protein has four high-affinity calcium-binding sites  $(K_D \sim 10^{-6} \text{ M})$  and the bovine intestinal protein two such sites<sup>5,6</sup>. The intestinal calcium-binding proteins are soluble species, located in the cytoplasm of the absorptive cells<sup>7</sup>. Their synthesis requires the presence of  $1\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) which, when given to a rachitic rat, produces an immediate increase in intestinal absorption of calcium, followed after a delay of several hours by the production of calcium-binding protein8. The protein thus cannot be involved in the initial translocation of calcium across the gut wall but could play a part in later stages of calcium metabolism. Alternatively, it could act as an intracellular calcium buffer, as the calcium-sensitive component of a transport molecule or as some other calcium-controlled regulator of absorptive cell

Although the three-dimensional structures of several calcium-binding proteins have been determined, of these only carp parvalbumin is believed to be involved in the regulation of intracellular calcium3. Parvalbumin contains two 29-amino acid calcium-binding domains, each consisting of a loop of 12 residues flanked by two  $\alpha$ -helices approximately at right angles to each other  $^{10,11}$ . This helix-loop-helix conformation is termed  $^{11}$ an 'EF hand' (see Fig. 1 of ref. 12 for derivation of the name). Based on an analysis of the key structural features of the two EF hands in parvalbumin, Tufty and Kretsinger<sup>12</sup> identified 16 amino acids in the 29-residue sequence that they believed were essential to the formation of a calcium-binding EF hand. They applied this 16-residue test sequence to a wide range of protein

sequences, and predicted that EF hands would be found in troponin C and other muscle proteins<sup>12</sup>. Subsequently this prediction was generalized to: intracellular calcium-modulated proteins contain EF hands and conversely, any protein which contains an EF hand is calcium modulated<sup>3,13,14</sup>.

This hypothesis has attracted broad interest but has not been subjected to a structural test. We present here the three-dimensional structure of a vitamin D-dependent calcium-binding protein from bovine intestine (ICaBP), which from its amino acid sequence6 is predicted to contain one, and possibly two, EF hand(s) (see Fig. 1). This protein indeed contains two helixloop-helix calcium-binding domains, resembling the EF hands in parvalbumin in both internal conformation and relationship to one another, but differing from them in detail.

#### X-ray analysis

ICaBP (minor A form; see Fig. 1) was purified115 from bovine intestinal mucosa to electrophoretic homogeneity and crystallized at pH 8.7-8.9 (ref. 16, Table 1). Derivatives for multiple isomorphous replacement phasing were prepared by soaking crystals in solutions containing various heavy-atom reagents and X-ray data were collected using both screened precession films and a Syntex P21 diffractometer, as summarized in Table 1. The single site in the Nd3+ derivative was located by a difference Patterson synthesis; sites in subsequent derivatives were determined by a combination of difference Patterson and crossphased difference Fourier syntheses<sup>17</sup>. Heavy-atom refinement was carried out using a program based on the ideas of Blow and Crick<sup>18</sup>, and of Matthews<sup>19</sup>. Statistics for the final cycle of refinement are shown in Table 1.

The native electron density map calculated at 2.3-A resolution using the multiple isomorphous replacement phases shows most of the molecular boundary and the general course of the polypeptide chain but appears noisy. To increase the signal-tonoise ratio, we applied a density modification procedure based on those of Schevitz et al.<sup>20</sup> and of Agard and Stroud<sup>21</sup>. This approach imposes two constraints: non-negativity of the electron density and uniformity (at a low level) of the electron density in solvent regions. Although only 35% of the unit cell is solvent, compared with 82% in the tRNA crystals used elsewhere20, this proved a useful approach. Density modification

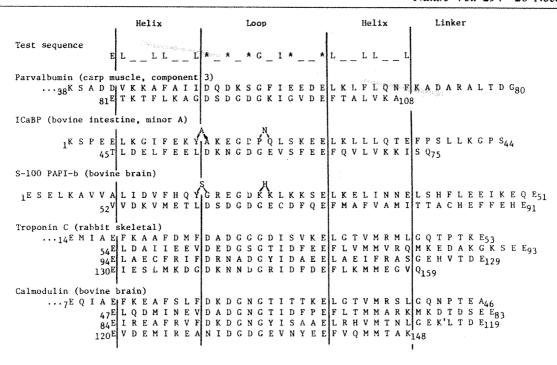


Fig. 1 Sequences of some calcium-binding proteins, with EF hand test sequence. The test sequence represents residues suggested from earlier studies to be critical to the EF hand structure<sup>12</sup>. In the test sequence, \* indicates an oxygen-containing residue (D, E, N, Q, S, T), L indicates a hydrophobic residue (L, V, I, F, M), I indicates a hydrophobic residue with isoleucine preferred, G is glycine, and an underscore indicates that any amino acid may appear in that position. Insertions and deletions have been postulated to maximize homology between sequences. N-terminal portions of parvalbumin<sup>34</sup>, troponin C<sup>35</sup> and calmodulin<sup>36</sup> have been omitted. The 'major' (native) form of ICaBP has the sequences Ac-Ser-Ala-Lys-minor A. Amino acid code: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; K', trimethyllysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. S-100 PAPI-b (bovine brain) sequence was from ref. 37; that of ICaBP, ref. 6.

Table	Table 1 Statistics for multiple isomorphous replacement phasing of ICaBP						
	Native	NdCl <sub>3</sub> (1 mM; 2-day soak)	KAu(CN) <sub>2</sub> (5 mM; 3-14-day soak)	K <sub>2</sub> Pt(CN) <sub>4</sub> (5 mM; 3-day soak)			
Unique reflections	3,023	3,031	3,124	2,601			
Scaling R	0.0687	0.0795	0.0754	-			
Film data resolution (Å)	2.3	2.3	2.3				
Diffractometer data resolution (Å)	2.9	_	3.4	2.7			
Reflections used	2,157*	2,025	2,051	1,532			
No. of sites	•	1	2	10			
Occupancy (electrons)		19+	45, 43	10, 12, 16, 15, 13 11, 8, 12, 7, 7			
R <sub>e</sub>		0.3441	0.5770	0.4098			
R <sub>K</sub>		0.0718	0.1444	0.1553			
E∕Î <b>f</b>	Overall 0.7642	0.7909	0.6018	1.0826			
A.D. residual		2.00	1.53	12.33			
ñ, final refinement cycle	0.6446						
n, phasing of native map	0.6109						
$\bar{m}$ , after density modification	0.7872						

$$\begin{split} R_c &= \frac{\Sigma \|F_{p \text{H}_{obs}}\| - \|F_{p \text{H}_{obs}}\|}{\Sigma \|F_p\| - \|F_{p \text{H}_{obs}}\|}, \text{ summed over centric reflections} \\ R_K &= \frac{\Sigma \|F_{p \text{H}_{obs}}\| - \|F_{p \text{H}_{obs}}\|}{\Sigma \|F_{p \text{H}_{obs}}\|}, \text{ summed over all reflections} \end{split}$$

Native crystals were grown from 65-80% saturated  $(NH_4)_2SO_4$  in 0.02 M Tris pH8.7-8.9, and stored in 90% saturated  $(NH_4)_2SO_4$ . Heavy-atom solutions contained the appropriate reagent in 90% saturated  $(NH_4)_2SO_4$ . The crystals are space group  $P2_12_12$ ; unit cell dimensions are:  $a=56.2\pm0.1$ ,  $b=42.6\pm0.1$ ,  $c=29.2\pm0.1$  Å. There is one molecule per asymmetric unit and a solvent content of 35%. Films were scanned on an Optronics P200 rotating drum densitometer and scaled together using the CORRELATE program of G. N. Reeke's ROCKS computing package. Diffractometer data were scaled to the film data; to compensate for absorption effects, different scale factors were permitted for different ranges of the diffractometer setting angles  $\phi$  and  $\chi$ . Intensities were placed approximately on an absolute scale using a Wilson plot. Derivative scale factors were adjusted to eliminate peaks and holes at heavy-atom sites on a native electron density map. Anomalous dispersion data for all derivatives were used in phasing.  $Nd^{3+}$  and  $Au(CN)_2^-$  positions and occupancies were refined phasing on all three derivatives but holding  $Nd^{3+}$  and  $Au(CN)_2^-$  parameters fixed. Effective isotropic temperature factors (Bs) were set to values of 10–15 and not refined.

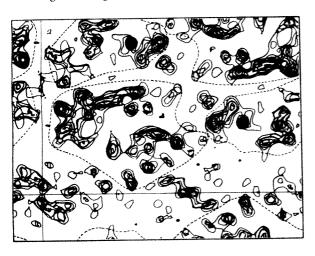
A.D. residual = 
$$\left[\frac{\sum w(AD_{obs} - AD_{calc})^2}{\sum wAD_{calc}}\right]^{-1/2}$$

<sup>\*</sup> Very weak native reflections and those with less than two good derivative measurements (counting the two members of a KAu(CN)<sub>2</sub> anomalous pair as two derivatives) were not used in phasing. Only the 2,080 reflections between 9.09 and 2.33 Å resolution were used for heavy-atom refinement, but all 2,157 reflections were used to phase the native map.

<sup>†</sup> Net occupancy. Nd<sup>3+</sup> replaces Ca<sup>2+</sup> (18 electrons), so the total Nd<sup>3+</sup> occupancy would be  $\sim$ 37 electrons.

increased the figure of merit from 0.61 to 0.79 and improved the clarity of the electron density map (compare the representative electron density sections before modification [Fig. 2a] with those after [Fig. 2b]). One or two spurious breaks in the polypeptide chain were introduced, but in most regions the modified map proved easier to interpret.

A Kendrew-Watson molecular model of ICaBP was constructed from the modified map using a Richards optical comparator<sup>22</sup>. The polypeptide backbone is shown in Fig. 3, based on the  $\alpha$ -carbon positions measured from this model. In most places, the course of the main chain is clear, although the exact conformation in some of the non-helical regions remains to be confirmed by crystallographic refinement. The register of the amino acid sequence<sup>6</sup> with the map is firmly established by the placement of the four prolines, all of which appear as flattened regions of high electron density in the main chain, and



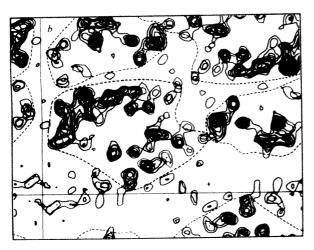


Fig. 2 Section through ICaBP electron density, from z = 0.375 to z = 0.425, before (a) and after (b) density modification. The density modification procedure was as follows: a probable molecular envelope was determined by inspection of the initial map, and all electron density values outside the envelope were considered to be in solvent regions and were set to a small constant value. Within the envelope, negative values were multiplied by 0.1 (to flatten out holes without creating sharp edges) and peaks above a certain maximum (slightly higher than the largest value on the original map) were truncated to that maximum. To reduce artefacts due to sharp edges, each point just outside the molecular envelope or just within the rim of a truncated peak was set to the average of itself with the 26 surrounding points. Inverse Fourier transformation of the modified map produced a new set of structure factors, whose phases were combined with the original phases using the method of Bricogne<sup>38</sup>. The combined phases were used with the observed amplitudes to produce a new map. After four cycles, the envelope was adjusted as indicated by the resulting map and applied to the original map. Seven cycles were then carried out using the revised envelope (indicated by dotted lines).

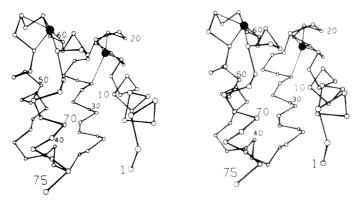


Fig. 3 Stereo view of the ICaBP  $\alpha$ -carbon backbone, with the two bound calcium ions.  $\bigcirc$ ,  $\alpha$ -carbons;  $\blacksquare$ , calcium ions.

by very clearly defined density for all aromatic side chains. The density is not consistent with an earlier sequence<sup>23</sup>.

### Overall structure of ICaBP

ICaBP is a globular protein of approximate dimensions  $25 \times$  $30\!\times\!30\,\mbox{\normalfont\AA}.$  It consists of four helices, designated I–IV and comprising residues 2-14, 24-35, 46-54 and 63-75, respectively, wrapped around the usual hydrophobic core of a soluble protein. There are hydrophobic contacts between side chains from all pairs of helices, and between the II-III linker and helices II and III. In addition to the four main helices, residues 36-41 are approximately helical, with hydrogen bonds formed between 36-40 and 38-41. Even neglecting this region, the helix content is ~63%, higher than indicated by circular dichroism studies (40±5% for bovine ICaBP (M. Jibson, unpublished results); 30% for the closely homologous porcine protein<sup>24</sup>). Regions I, II and III are standard  $\alpha$ -helices whereas IV begins with four residues of  $3_{10}$  helix and then becomes  $\alpha$ -helical. The region between helices III and IV is a calcium-binding loop of the EF hand type. Between I and II is a slightly longer loop which also binds calcium and may be described as a variant EF hand (see below). If the linker region between II and III is neglected, there is an approximate 2-fold axis relating the I-II and III-IV domains, illustrated by the view down this axis shown in Fig. 4a.

The single tyrosine, Tyr 13, lies on the surface of the molecule, making a probable hydrogen bond to Glu 35, thus tying helices I and II together. Birdsall *et al.*<sup>25</sup> proposed, on the basis of NMR work, that the Tyr lies in a hydrophobic pocket involving one Ile and two Phe. We find Tyr 13 to be in a very shallow depression formed by Ile 9, Leu 30, Leu 31, Thr 34 and Glu 35. Phe 10, Phe 36 and Phe 66 are within 11 Å of the Tyr, which is close enough for some interaction. Fluorescence measurements demonstrate complete resonance energy transfer from all five Phe residues to the Tyr. For the two Phe further than 10–12 Å from the Tyr, energy transfer presumably involves an inter-Phe relay system; no Phe is more than 11 Å from the rest.

#### EF hands in ICaBP

The calcium-binding loop between helices III and IV is extremely similar to the EF hand prototype found in parvalbumin. The calcium is coordinated by side-chain oxygens from Asp 54, Asn 56, Asp 58 and Glu 65 and by the main-chain carbonyl from Glu 60. Both carboxyl oxygens of Glu 65 are ligands, so that there are six protein oxygens surrounding the calcium ion at distances (unrefined) of 2.2–2.6 Å. The calcium in the EF loop of parvalbumin has a water molecule in its coordination sphere; the ICaBP III–IV calcium is clearly exposed to solvent, and initial refinement suggests that a solvent molecule is a seventh ligand. Helices III and IV are at an angle of 121° with a minimum inter-axis distance of 8.06 Å, compared with 102° and 7.63 Å in the parvalbumin EF hand. Fitting the α-carbons of the ICaBP III–IV domain to those of the parvalbumin EF region

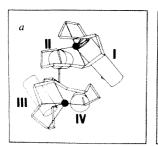
Table 2	ICaRP.	compared with parvalbumin

a Inter-helix angles an	d distances				
_	Parvalbumin			ICaBP	
Helix pair	Inter-helix angle	Minimum distance (Å) between helix axes	Helix pair	Inter-helix angle	Minimum distance (Å) between helix axes
EF hand helices					
C-D	103°	8.37	I-II	121°	8.06
E-F	102°	7.63	III–IV	121°	8.55
Other helices in cont	act				3122
A-B	155°	5.06			
A-D	150°	10.35			
A-E	120°	9.34			
B-F	73°	7.80			
C-F	119°	9.35	I-IV	123°	10.87
D-E	11 <b>7</b> °	9.59	II–III	121°	9.90
F hand α-carbon fi	ts	ħ	II–IV	21°	11.52
Segr	ments compared		No. of atoms	r.m.s. discrepancy (Å)	r.m.s. discrepancy 14 loop atoms (Å)
CD (40-69) vs. EF (7	(9–108)		30	1.86*	0.93
CD (40-71) vs. I-II (	3-36)†		32	2.86	1.99
EF (78-108) vs. I-II	(2-34)†		31	2.75	2.11
CD (43-71) vs. III-IV	V (46–74)		29	3.25	1.41
EF (82-108) vs. III-I	V (46–72)		27	2.40	1.26
$I-II$ $(6-36)\dagger$ vs. $III-IV$	'		29	3.33*	2.43
CD, EF (38–71, 82–1	08) vs. I-II, III-IV (1-	-36†, 46–72)	61	3.07	

a, Angles and minimum inter-axial distances between all pairs of helices which are in contact. The inter-helix angle is calculated by considering the helix axes as vectors, and is  $0-90^{\circ}$  for parallel,  $90-180^{\circ}$  for antiparallel helices. The distance is the minimum distance between infinite lines along the helix axes; the closest approach of the finite helices may be greater. b, Agreement between  $\alpha$ -carbon positions of indicated segments after rotation and translation of one set of coordinates so as to minimize  $\Sigma d_i^2$ , where  $d_i$  is the distance between the ith  $\alpha$ -carbons of the two segments after rotation and translation. Parvalbumin coordinates were supplied by the Protein Data Bank<sup>33</sup>.

results in an average (r.m.s.) discrepancy of 2.40 Å for the entire domain and 1.26 Å for the calcium-binding loops alone; the corresponding values for CD as opposed to EF are 1.86 and 0.93 Å (Table 2).

However, the I-II loop (two residues longer) is rather different. The insertion of Ala 14 means that the first predicted calcium ligand, Ala 15, is on the outside rather than the inside of helix I, and of course the alanine side chain contains no oxygen. This lack is accommodated by using the main-chain carbonyl of Ala 15 as a ligand. The loop is further rearranged about the second and third calcium ligands, Glu 17 and Asp 19, so that they also coordinate Ca<sup>2+</sup> with their main-chain carbonyls rather than their side chains. Following the second insertion of Asn 21 (or Pro 20), the loop adopts essentially the normal EF hand conformation. The main-chain carbonyl of Glu 22 and the two carboxyl oxygens of Glu 27 constitute the remaining calcium ligands, giving a total of six protein oxygen ligands. Ca-O distances (unrefined) are 2.2-2.6 Å. Due to the different conformation of the N-terminal portion of the loop, the I-II calcium



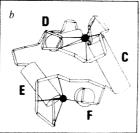


Fig. 4 α-Carbon backbone and calcium ions viewed down the approximate intramolecular 2-fold axis of (a) ICaBP and (b) parvalbumin, residues 38-108. Helices are represented by cylinders and other polypeptide chains as a folded ribbon. ●, Calcium ions.

is much less accessible to solvent than the III-IV calcium, and it is unlikely that a solvent molecule is a seventh ligand.

The stretches of calcium-binding loop from residues 22-25 and 60-63 run antiparallel to one another and are joined by a hydrogen bond between Leu 23 and Val 61, across the approximate 2-fold axis, resembling a small piece of  $\beta$ -sheet. This arrangement also occurs in parvalbumin, between the CD and EF loops. The calcium ions are separated by 11.9 Å in parvalbumin and by 12.5 Å in ICaBP.

In both the conformation of the two calcium-binding domains and their disposition with respect to each other, ICaBP thus resembles the C-terminal two-thirds of parvalbumin (Fig. 4). If the II-III linker and the two inserted residues in the I-II loop are omitted, a fit of the remaining 61 ICaBP  $\alpha$ -carbons to the corresponding parvalbumin  $\alpha$ -carbons gives a r.m.s. discrepancy of 3.07 Å. Compare, for example, the 5.7 Å r.m.s. discrepancy between 60-residue segments of T4 lysozyme and hen egg-white lysozyme, and the 0.9 Å (for a 40-residue stretch) to 1.5 Å (for 80 residues) discrepancy between myoglobin and haemoglobin  $\beta$ -chain<sup>27</sup>.

Despite this resemblance, there are significant differences between the conformations of ICaBP and parvalbumin. Helices II and IV in ICaBP are in contact, whereas the corresponding helices D and F in parvalbumin are not (Table 2). Inter-helix angles differ and are generally more regular in ICaBP (Table 2). Parvalbumin has an extended D-E linker, a bend in the D helix and a straight F helix, whereas the ICaBP II-III linker is highly folded, helix II is straight and there is a bend in helix IV. These differences in helix spacing and linker folding are only to be expected, because if ICaBP were to adopt the parvalbumin conformation, it would have a large hole in its 'underside' which is filled by the A and B helices in parvalbumin. ICaBP helix III is shorter than parvalbumin helix E and IV is slightly longer than F. Finally, the I-II and CD loops differ: the latter is a normal EF hand calcium-binding loop whereas the former is a variant. Thus, although ICaBP exhibits the same overall helix-loophelix structure found in parvalbumin, the disposition of the

<sup>\*</sup>The transformation relating these segments, expressed as a screw operation, is 172°, -0.78 Å for parvalbumin, and 168°, -0.32 Å for ICaBP, that is, in both cases the two segments are related by an approximate 2-fold axis.

<sup>†</sup> Asn 21 omitted. Ala 14 used in 14 residue loop but omitted from helix-loop-helix comparison.

helices with respect to each other, both within one domain and between domains, is clearly different. This may hinder attempts to search for EF hands by application of molecular replacement techniques to crystals of other calcium-regulated proteins such as calmodulin and troponin C.

#### Calcium-binding to ICaBP

We believe that the structure described here represents ICaBP having both high-affinity sites fully loaded with calcium and no significant occupancy of low-affinity calcium sites. Weakly bound calcium was removed by dialysis against distilled water. The presence of calcium in the high-affinity sites is evidenced by peaks near both sites on a native anomalous difference Fourier (the two highest peaks on the map); and, in I-II, by the presence of a peak at the calcium position on the native electron density map. Site III-IV does not have a peak at exactly the calcium position, but as it is a heavy-atom site (for the Nd<sup>5+</sup> derivative), distortions in this region are to be expected. Full occupancy of the high-affinity sites is suggested by the good definition of the polypeptide chain in both calcium-binding loops and by the absence of changes in structure amplitudes on soaking crystals in 10 mM CaCl<sub>2</sub>. NMR<sup>25</sup> and trypsin digestion<sup>28</sup> experiments reveal a substantial conformational change in the protein on removal of one or both calcium ions. This conformational change is unfortunately sufficient to disintegrate ICaBP crystals soaked in 1.0 mM EDTA, and attempts to grow crystals in the absence of calcium have been unsuccessful. Thus, the conformational changes which occur on release of one or two calcium ions are unknown; they might involve only small rearrangements of the calcium-binding loops, or motion of the flanking helices with respect to each other, or motion of the two calcium-binding domains.

The two calcium-binding sites differ in their calcium affinities, indicated by the removal in EDTA (solution of 1:1 EDTA/ICaBP) of only one calcium (C. S. Fullmer, personal communication). The relative affinities for Tb<sup>3+</sup>, a fluorescent model for Ca<sup>2+</sup>, also differ, by at least a factor of 20 (ref. 26). It is apparent from the X-ray structure that the III-IV site is more exposed to solvent than the I-II site; this agrees with the observation that, in a crystal soaked for 2 days in 1.0 mM NdCl<sub>3</sub>, Nd<sup>3+</sup> displaces the III-IV but not the I-II Ca<sup>2+</sup>. We suggest that the I-II site may be a structural site, saturated with calcium in all physiological conditions, whereas the III-IV site is a regulatory site which binds or releases calcium as intracellular calcium levels vary.

#### Implications for the structure of other calcium-binding proteins

ICaBP is a member of the calmodulin family of calcium-binding proteins, for which it is proposed that an ancestral four-domain calcium-binding protein was produced by successive gene duplications<sup>29</sup>. Calmodulin and troponin C have retained this type of structure, and bind four calcium ions per protein molecule. Some other members of the family have lost the calciumbinding ability of one or more of the domains. In parvalbumin, the first domain was deleted entirely and the second, the AB domain, no longer binds calcium; in ICaBP, two domains were deleted and the N-terminal I-II domain was modified by the insertion of two residues, while retaining its calcium-binding

The S-100 PAPI-a and PAPI-b proteins belong to the same branch of the calmodulin family as ICaBP; divergence seems to have occurred after the modification of the I-II loop (Fig. 1 shows the PAPI-b sequence; PAPI-a is 58% homologous to PAPI-b overall and almost identical with respect to the calciumbinding loops<sup>30</sup>). The S-100 proteins are thus expected to contain one variant and one normal EF hand, arranged similarly to those in ICaBP.

The calcium-binding characteristics of PAPI-b, however, are more complex than those of ICaBP. Two Ca<sup>2+</sup> are bound (per monomer) at pH 8.3 but only one at pH 7.6. Furthermore, the

presence of K+ inhibits binding of calcium to the high-affinity sites31. PAPI-b contains three basic side chains Lys-His-Lys in positions corresponding to Pro-Asn-Gln in the I-II loop of ICaBP (Fig. 1). It is likely that titration of the histidine is responsible for the pH dependence of calcium-binding, and the overall affinity of this site for cations may be lowered relative to ICaBP by the proximity of additional basic side chains.

Kretsinger and Barry<sup>32</sup> constructed a model for the fourdomain calcium-binding protein, troponin C, from four EF hands, by first pairing two hands as in the parvalbumin CD plus EF region, duplicating the pair and placing the non-calciumbinding ends of the pairs together so that a quasi 2-fold axis perpendicular to the one within each pair relates the two pairs. The molecule then exhibits quasi-222 symmetry. A similar model may be constructed for other four-domain proteins such as calmodulin. This structural prediction is supported by our results: the conformation of the ICaBP III-IV domain proves that the normal EF hand occurs other than in parvalbumin, and the stability of pairs of EF hands is demonstrated by the maintenance of such a pair, related by an approximate 2-fold axis, in ICaBP even when one calcium-binding loop has been appreciably modified.

The overall conformations of proteins in the calmodulin family are thus determined by the preferred formation of EF hands and pairs of hands, which may be packed together into higher aggregates. Differences in function are made possible by variation in the conformation of the calcium-binding loops, in the disposition of helices with respect to each other, and/or by differences in the linker regions. The conformation of the linkers, in particular, is quite free to evolve as required for interaction with other molecules, without disturbing the overall shape or calcium-binding abilities of the protein.

This determination of the ICaBP structure provides the first structural confirmation of the EF hand hypothesis, and also expands the definition of an EF hand, first to include structures whose helices are disposed rather differently from those in parvalbumin (for example, the ICaBP III-IV domain), and second to include structures with a modified calcium-binding loop such as the ICaBP I-II domain. These latter variant EF hands are not necessarily detectable from sequence comparisons; if the existence of two insertions is not recognized, only 8 out of 16 residues of the I-II domain match the EF hand test sequence. Hence the recognition of such variants in other proteins (except for those such as the S-100 proteins which are clearly closely related to ICaBP) will require the determination of their three-dimensional structures.

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# LETTERS TO

### Absorption of $\gamma$ rays in active galaxies as a test of the jet hypothesis

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Powerful X-ray emission is a common feature of active galaxies, some of which have also been detected as  $\gamma$ -ray sources<sup>1-5</sup>. The observed high luminosities, coupled with reports of variability on time scales of days or less at X-ray energies<sup>6</sup> and of 1 yr or less at  $\gamma$ -ray energies<sup>3</sup>, suggest that both emissions may originate in the same volume deep within the nucleus of the galaxy. Under this assumption we investigate here the attenuation of  $\gamma$  rays as a result of pair-production in photon-photon collisions with X rays and show that whilst Seyfert galaxies are transparent to  $\gamma$ radiation up to ~1 GeV, QSOs and BL Lac objects are generally opaque about 1 MeV. However, the detection of  $\gamma$  rays from these latter classes of objects may be explained if beaming of the  $X-\gamma$  emission is assumed.

The relevance of photon-photon absorption to specific astronomical sources has been discussed by several authors<sup>7</sup> Following their formalism, a photon of energy  $E_x$  may collide with a photon of energy  $E_{\gamma}$  and produce an electron-positron pair provided that

$$E_{\mathbf{X}}E_{\mathbf{y}} \ge \frac{2(mc^2)^2}{(1-\cos\theta)}\tag{1}$$

where  $mc^2 = 0.511 \text{ MeV}$  is the rest mass energy of the electron and  $\theta$  the angle between the directions of motion of the two photons. For fixed values of  $E_{\gamma}$ , the pair production crosssection  $\sigma(E_X)$  rises steeply from zero at the threshold energy  $E_X = E_s$  to a maximum value  $\sigma_{\text{max}} = 1.7 \times 10^{-25} \text{ cm}^2$  at  $E_X = 2E_s$ and then falls off as  $E_x^{-2}$  for energies  $E_x \gg E_s$ . The optical depth for this process can be written as:

$$\tau = R \int \sigma(E_{\mathbf{X}}) N_{\mathbf{X}}(E_{\mathbf{X}}) \, \mathrm{d}E_{\mathbf{X}} \tag{2}$$

where the cross-section  $\sigma$  and the density of X-ray photons per unit energy  $N_x$ , are assumed to be constant throughout the source region of size R. In the first instance we consider the case of isotropic emission and for simplicity set  $\theta = \pi/2$ . Furthermore within the limits of accuracy required by this calculation it is sufficient to approximate  $\sigma(E_x)$  by a rectangular function of height  $\sigma_{\text{max}}$  and width 2.5  $E_{\text{s}}$ . Consequently, the optical depth may be written as

$$\tau = R\sigma_{\text{max}}N_{\text{X}}(2E_{\text{s}})2.5E_{\text{s}} \tag{3}$$

To estimate  $N_X$  we use the X-ray luminosity and write

$$N_{\rm X}(2E_{\rm s}) = f(\alpha)L_{\rm X}\frac{(2E_{\rm s})^{-\alpha}}{4\pi R^2 c}$$
 (4)

where  $\alpha$  is the source photon spectral index at X-ray energies.

For  $L_{\rm X}$  taken over a fixed energy band (generally 2-10 keV),  $f(\alpha)$  is a slowly varying function of  $\alpha$ . Introduction of numerical values in equation (3) leads to the final expression

$$\tau = 2.5 \times 10^{-28} f(\alpha) \frac{L_{\rm X}}{R} (2E_{\rm s})^{-\alpha+1}$$
 (5)

with  $L_X$  in erg s<sup>-1</sup>, R in cm, and  $E_s$  in keV. For convenience we have taken  $\alpha = 1.6$  for each of the sources considered. This value represents a reasonable description of the observed spectra of Seyfert galaxies<sup>11</sup> and quasars<sup>12</sup>. It is less precise for BL Lac objects, whose spectral variability makes the choice of an average  $\alpha$  value difficult<sup>13</sup>. However, the function  $f(\alpha = 1.6)$ only varies between 0.34 and 0.37 for the different energy bands over which the X-ray luminosities are calculated. Table 1 lists the values of  $L_X$  and  $R = c \Delta t$  together with the optical depths  $\tau$ for a range of  $\gamma$ -ray energies. Note that R represents an upper limit on the size of the X-ray region and consequently a lower limit on  $\tau$ , because, in many cases, the  $\Delta t$  values are related to periods between observations rather than to measured changes in the X-ray emission.

Table 1 shows that Seyfert galaxies are transparent to their  $\gamma$ radiation up to energies of ~1 GeV. The size of the emitting region is probably not very different from one Seyfert to another so that the value of  $\tau$  will ultimately depend on the X-ray luminosity of the object in question. Consequently, the less bright X-ray sources could be the best candidates for γ-ray emission. As Type II Seyferts have an average X-ray luminosity which is approximately two orders of magnitude lower than that of type I Seyferts 14,15 we suggest that Type II objects should be primary targets for future y-ray observations. In contrast it is clear that quasars and BL Lac objects must be effectively opaque to y rays of a few MeV or more, if isotropic emission of both the X and  $\gamma$  rays is assumed. Many of the electron-positron pairs produced in this absorption process will eventually annihilate to produce 511 keV radiation, which is no longer absorbed by the X-ray photon field and can probably escape depending on the matter density present close to the emission region. If the positrons are slowed down such that the line is not smeared out by the relativistic kinematics, we may expect that these objects should be characterized by a weak 511 keV flux. Because in the case of the QSO 3C273 γ rays have been detected<sup>5</sup> in the energy range 50-500 MeV, we must conclude that either the X- and  $\gamma$ -ray emission regions do not coincide or that anisotropic, highly collimated radiation is a characteristic of this active galactic nucleus. The degree of continuity in the spectral emission between the hard X-ray and the low energy  $\gamma$ -ray fluxes suggests that the possibility of beamed radiation is more likely. In the simplified model of jets consisting of streams of relativistic particles, equation (1) shows that as  $\theta$  approaches zero, the threshold energy,  $E_s$ , tends towards infinity and the absorption process of y rays becomes inhibited for higher and higher energies. For the case of a homogenous diverging beam with opening angle  $\theta_0$ , the limiting photon energy which is absorbed

Table 1 Photon-photon absorption for various extragalactic sources

						Photon-	photon optical	depth as a f	unction of
		$L_{\mathbf{X}}(2-10 \text{ keV})$		R			γ-ray	energy	
Source	Type	$(erg s^{-1})$	Ref.	(cm)	Ref.	1 MeV	10 MeV	$10^2 \mathrm{MeV}$	$10^3  \mathrm{MeV}$
NGC7469	<b>S</b> 1	$5.4 \times 10^{43}$	6	$5.2 \times 10^{15}$	6	0.02	0.08	0.30	1.20
NGC4151	S1	$6.2 \times 10^{42}$	22	$7.0 \times 10^{14}$ *	22	0.02	0.06	0.26	1.03
NGC6814	S1	$5.4 \times 10^{42}$	6	$6.0 \times 10^{14}$	23	0.02	0.07	0.26	1.04
NGC3783	S1	$2.3 \times 20^{43}$	6	$5.2 \times 10^{15}$	. 11	0.01	0.03	0.13	0.51
MKN509	S1	$2.5 \times 10^{44}$	24	$3.1 \times 10^{16}$	24	0.01	0.06	0.23	0.94
MCG8-11-11	S1	$8.3 \times 10^{43}$	25	$7.8 \times 10^{16}$	25	0.002	0.008	0.03	0.12
NGC2992	\$2	$1.8 \times 10^{43}$	6	$7.8 \times 10^{15}$	6	0.004	0.02	0.07	0.27
NGC5506	S2	$1.3 \times 10^{43}$	26	$7.8 \times 10^{15}$	26	0.003	0.01	0.05	0.19
NGC526A	S2	$7.4 \times 10^{43}$	6	$7.8 \times 10^{15}$	6	0.02	0.07	0.28	1.10
NGC7582	\$2 \$2	$5.3 \times 10^{42}$	6	$5.7 \times 10^{16}$	27	$1.7 \times 10^{-4}$	$6.8 \times 10^{-4}$	0.027	0.01
MCG5-23-16	\$2 \$2	$2.2 \times 10^{43}$	28	$1.0 \times 10^{16}$	28	0.004	0.02	0.06	0.26
3C273	QSO	$1.1 \times 10^{46}$	6	$1.3 \times 10^{15} †$	6	15.6	61.9	246.5	981.2
2SO241+622	QSO	$1.0 \times 10^{45}$	12	$5.2 \times 10^{15}$	6	0.35	1.41	5.6	22.30
OX169	QSO	$1.1 \times 10^{44}$ ‡	23	$1.8 \times 10^{14}$	23	1.24	4.93	19.62	78.12
MKN421	BL Lac	$2.2 \times 10^{44}$	6	$2.6 \times 10^{15}$	6	0.16	0.62	2.46	9.81
PKS2155 - 304	BL Lac	$6.4 \times 10^{45}$ §	6	$6.0 \times 10^{14}$	29	19.6	78.1	0.7	1,236.9
3G66A	BL Lac	$1.8 \times 10^{46}$	30	$5.7\times10^{15}$	30	6.07	24.16	96.17	382.9

<sup>\*</sup> X-ray variability also reported with  $\Delta T = 700$  s (ref. 23) but of small statistical significance.

by photon-photon pair production can be written as  $E_{\gamma} \sim$  $\sqrt{2} mc^2 (1-\cos\theta_0)^{-\frac{1}{2}}$ . Thus to observe  $\gamma$  rays from 3C273 would require a beaming angle of few degrees or less. More realistic jet models 16-20 involving relativistically moving plasmas, present a more complex picture. First the absorption calculation must be made in the rest frame of the flow where the emission is isotropic. Second, corrections must be applied for the combined relativistic effects of Doppler blueshifting of the  $\gamma$ -ray photons and of beaming which is responsible for increasing the source luminosity. The overall effect is to reduce the absorption of  $\gamma$ 

rays in emitters of beamed radiation.

It has been suggested 19,20 that the sequence of extragalactic objects, radio-quiet quasars, radio-loud quasars and blazars (optically violent variable quasars and BL Lac objects) correspond to sources associated with relativistic jets, which are received at progressively smaller angles to their axes. In this picture, the nucleus may be considered to have an isotropic steady and unpolarized continuum which in the optical range is responsible for the emission lines and, in addition, a variable, strongly polarized source associated with a jet and beamed in a cone of small solid angle. The beamed component progressively dominates the isotropic emission as the angle between the observer and the jet axis decreases. Under this hypothesis, BL Lac objects probably represent the extreme case of alignment with the beam, so that the isotropic component and, consequently, the emission lines are completely swamped. Of the three quasars discussed here, two are classified as radio loud quasars<sup>21</sup> and probably represent a less extreme case of alignment with the direction of the Earth. We would therefore expect both classes of objects to be  $\gamma$ -ray sources as along the beam direction little photon-photon absorption is expected. Conversely sources for which the beam is not directed towards the observer would not be detected as  $\gamma$ -ray emitters. If this is true, a future γ-ray survey of extragalactic objects should be carried out to test the possible correlation between the  $\gamma$ -ray spectral intensity and the angle of observation with respect to the jet axis. We conclude that y-ray observations of extragalactic objects could not only test the beaming hypotheses but also support the argument that the classification of 'different' types of active galaxies is related to their orientation with respect to the Earth, rather than any intrinsic differences in their internal structure.

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### Are coronal loops stable?

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The solar corona is now recognized as a highly inhomogeneous plasma that comprises a complex network of individual arch and loop-like structures1. This has led to the recent idea that the isolated 'coronal loop' may form a basic building block of quiescent solar and stellar atmospheres2.3. Theoretical , however, suggest that isolated loops are violently studies4.5 unstable and hence should collapse on the radiative time scale of the plasma. The simple fact that loops exist stably over long periods demonstrates the inadequacy of the current theory. We point out here that a crucial ingredient is missing from the theory and hence reconcile the existence of coronal loops with the disruptive effect of the radiative instability.

The central assumption in theoretical loop modelling is that dynamic effects can be neglected in the local energy balance<sup>6</sup>.

<sup>† 10%</sup> X-ray variability also observed on  $\Delta T = 610^3$  s (ref. 23).

 $<sup>\</sup>ddagger L_X$  in the range 0.5-4.5 keV

<sup>§</sup> Redshift measurement doubtful.

 $<sup>||</sup>L_X|$  in the range 0.2-3.5 keV.

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Hence it is postulated that an unknown heating mechanism supports the loop against energy losses by radiation and energy redistribution by heat conduction along the confining magnetic field lines. This leads quite naturally to a scaling law which, for the loop of length l, relates the plasma pressure P to the peak coronal temperature in the loop:

$$P = 2.1 \times 10^{-11} \beta^{1/2} T_0^{13/4} / l \text{ dyn cm}^{-2}$$
 (1)

where  $\beta$  is a number of the order of unity accounting for the shape of the heating function<sup>2</sup>. An important prediction of the quasi-static model therefore is that small, hot loops should be several orders of magnitude denser than larger, cooler structures. That such behaviour is confirmed observationally is very strong support for the quasi-static analysis.

Yet an astrophysical plasma hotter than 10<sup>5</sup> K may be unstable because the radiative loss rate decreases with increasing temperature. Field's stability criterion<sup>7</sup> for a uniform conducting plasma is

$$P < 6.1 \times 10^{-11} T^{13/4} / l \text{ dyn cm}^{-2}$$
 (2)

Although equation (2) is not strictly applicable to a non-uniform coronal loop, comparing equations (1) and (2) we anticipate that conduction may not be able to control the instability. Independent normal mode stability analyses in fact claim that the 'thermally isolated' loop (that is, a loop whose temperature gradient vanishes at the lower boundary) is radiatively unstable<sup>4,5</sup>. This result implies that if the loop is to exist at all it cannot be described by a quasi-static energy balance. Thus it has been speculated that a dynamic solution may exist in which the radiative instability drives periodic dynamic oscillations on the radiative time scale of the plasma4-6

This conclusion is disturbing for two main reasons: first, quiescent loops show little evidence of significant dynamical behaviour, nor are they observed to collapse on the radiative time scale of the plasma<sup>1-3</sup>. Second, detailed hydrodynamic models based on the same physics as the quasi-static analysis do not reveal oscillatory modes driven by the radiative  $instability^{8-10} \\$ 

In attempting to resolve this problem we have applied a standard normal mode analysis4 to a 'thermally isolated' loop (see also refs 5, 11). Two classes of lower boundary conditions are considered: in one case the base is held at a fixed chromospheric temperature  $(\delta T_b = 0)$ ; in the other the heat flux is assumed to vanish at the base  $(\delta F_b = 0)$ . As the former condition implies that the chromosphere can act as an infinite source or sink of heat while the latter implies that no heat is exchanged across the lower boundary9, we expect that a real loop must lie between these extremes. In either case, because the base of the loop cannot move freely  $^{12}$  ( $\delta v_{\rm b} = 0$ , where v is the velocity), the most unstable eigenmode is the first antisymmetric mode<sup>4</sup> symmetric modes tend to be stabilized by the rise in pressure which accompanies a temperature increase.

It can be proved that the loop is marginally stable in the case where the base temperature is held fixed. To see this, we note that the first antisymmetric eigenfunction given by

$$\delta T \propto T', \quad \delta v \propto T - T_{\rm b}$$
 (3)

where T' denotes the unperturbed temperature gradient, represents an analytic solution to the problem<sup>4</sup> corresponding to the case of zero growth. This result also establishes that the loop is unstable when  $\delta F_b = 0$ , as, by Sturm's oscillation theorem<sup>5</sup>, the eigenvalue λ (the negative of a normalized growth rate<sup>4</sup>) must decrease to satisfy the required boundary condition. However, the instability is physically insignificant if the amount of radiatively stable chromospheric material ( $T < 10^5$  K) at the foot of the transition region is sufficiently large. The growth time is then greater than any relevant time scale associated with the loop.

Figure 1 shows the first antisymmetric ( $\delta F_b = 0$ ) eigenvalue  $\lambda$ plotted against the ratio of chromospheric  $(T < 10^5 \text{ K})$  mass to coronal  $(T > 10^5 \text{ K})$  mass in the loop,  $N_{CH}/N_{CO}$ , for various base temperatures T<sub>b</sub>. A slight increase in chromospheric mass clearly produces a dramatic decrease in  $\lambda$ ; likewise the growth rate of the instability decreases. From the definition<sup>4</sup> of  $\lambda$  and

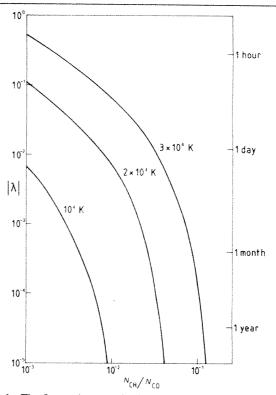


Fig. 1 The first antisymmetric eigenvalue  $\lambda$  (normalized growth rate) plotted against chromospheric mass  $(T < 10^5 \text{ K})$  for a range of base temperatures  $T_{\rm b}$ . The amount of chromospheric material  $N_{\rm CH}$ is expressed in units of the coronal mass  $N_{\rm CO}$ . On the right-hand scale actual growth times for the typical values  $T_0 = 2.5 \times 10^6 \text{ K}$  and  $l = 10^{9.5} \text{ cm}$  are shown.

the scaling law (1) we find (setting  $\beta = 1$ ) the actual growth time

$$\tau = 2.7 \times 10^{-5} \frac{l}{|\lambda| T_0^{1/4}}$$
 s (4)

which for the typical values  $T_0 = 2.5 \times 10^6 \text{ K}$  and  $l = 10^{9.5} \text{ cm}$ yields the growth times shown on Fig. 1. Values of  $\tau$  greater than a few weeks have no physical significance as the growth time exceeds the lifetime of a typical loop.

We conclude that the radiative instability is controlled essentially by the chromosphere: as more and more of the radiatively stable chromospheric plasma is included in the analysis the  $(\delta F_{\rm b} = 0)$  mode of instability tends towards the marginally stable case ( $\delta T_b = 0$ ). Very little chromospheric material is, in fact, required to counter the instability: between 1 and 10% of the coronal mass suffices. It follows that the rapid instabilities found previously<sup>4,5,11</sup> are simply a consequence of the neglect of the chromospheric influence.

Finally, we note that a complete solution to the problem requires the treatment of energy transport by radiative transfer in the chromosphere. Because only a minute fraction of the radiatively stable material available is required to quash the instability in the optically-thin approximation used above, we believe our conclusions will remain essentially unaltered by the inclusion of radiative transfer.

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# Dayside electron cyclotron harmonic emissions

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Observations of waves exhibiting an often complex frequency banding related to the electron gyrofrequency were first reported in 19701. Later observations2-5 showed that the spectral content of these electrostatic electron cyclotron harmonic waves is highly variable<sup>6,7</sup>, including single frequency emissions between the electron gyrofrequency  $\Omega_e$  and its first harmonic, multiple bands between consecutive harmonics of  $\Omega_e$ , and emissions in a single band far above the electron gyro-frequency<sup>8-10</sup>. These observations have led theorists to investigate both the linear instability which generates the waves 11-15 and various wave-particle 14,16 and wave-wave 17-18 interactions in which they may be involved. The consensus seems to be that at least two plasma components with different temperatures are required to destablilize electron cyclotron harmonic waves. We adopt this view here and test our present knowledge of electron cyclotron harmonic instabilities against the observed reality. To do this we use simultaneous observations of wave spectra and electron distribution functions, available in the GEOS 1 data from 25 August 1977 (see ref. 15). From these we present the wave event and then use the measured particle fluxes to derive a model distribution function, providing the input to a computer program which solves the plasma dispersion relation. From the computed temporal growth rates and group velocities, the total amplification of waves which are unstable within a limited volume of space is estimated.

The emissions observed by the \$300 wave experiment onboard GEOS 1 between 09.20 and 10.20 UT on 25 August 1977 are shown in Fig. 1. The spectrogram is derived from the large electric antenna 19 and consists of sets of 0-77 kHz sweeps of the stepped frequency analyser. The grey scaling is adjusted to the strongest signal in each sweep. The horizontal lines at 47.6 and 63.5 kHz are caused by the satellite clock pulses and are thus purely instrumental. The line  $f_{ce}$  indicates the electron gyrofrequency, which during the event was ~4.4 kHz. The variable high frequency emissions seen before 09.40 UT are known to be in the vicinity of the upper hybrid (or plasma) frequency. When the plasma frequency drops to around 30 kHz after 09.40 UT, signals appear between all gyroharmonics below the intensified plasma line. The signal at the second harmonic of the plasma frequency is believed to be (at least partly) instrumental, but it indicates a very strong signal at the fundamental. The emissions observed after 09.40 UT would belong to class 3 in the classification schemes of Gough et al.7 and of Hubbard and Birmingham<sup>6</sup>. The higher harmonics disappear at around 09.53 UT, while the  $3/2~\Omega_e$  emission persists until 10.00 UT. After this time, only the upper hybrid (or plasma) line remains. At 09.50 UT, the satellite was on the dayside (11.20 MLT) at  $L \sim 6.6$  and  $\sim 10^{\circ}$  magnetic latitude.

The importance of the cool plasma parameters for electron cyclotron harmonic instabilities is discussed in the accompanying paper by Horne *et al.*<sup>20</sup>. Low energy (0-500 eV) electron fluxes are measured by the S302 experiment onboard GEOS 1 (ref. 21), and information about the low energy electrons at 09.47 UT has been provided by J. Johnson, J. Sojka and G. Wrenn (personal communication). The main components have temperatures  $T_1 \approx 1 \text{ eV}$  and  $T_2 \approx 5 \text{ eV}$ , and the densities are

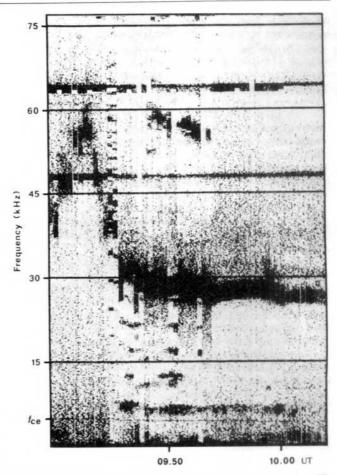


Fig. 1 Spectrogram emphasizing the wave event under study. The display begins at 09.20 UT the time scale is not uniform.

 $n_1 \approx 5.5 \times 10^6$  electron m<sup>-3</sup> and  $n_2 \approx 3.0 \times 10^6$  electron m<sup>-3</sup>. In addition, a component with temperature  $T_3 \approx 200$  eV and density  $5 \times 10^4$  electron m<sup>-3</sup> is observed. The flux of this component at a pitch angle of 80° is double the flux at 18°. This anisotropy and the slope of the energy spectrum match the low energy end of the distribution measured by the S310 experiment, although the flux values given by S310 are higher.

The particle experiment S310 provides pitch angle distributions and energy spectra in the range 0.35-22 keV (ref. 22). When the angle between the magnetic field and the spin axis is  $15^{\circ}-26^{\circ}$ , at least one of the four detectors with a narrow field of view ( $\pm 2^{\circ}$ ) passes through the loss-cone. In such cases even the very narrow loss-cone expected at geostationary altitudes can be resolved. At larger pitch angles, measurements are made by

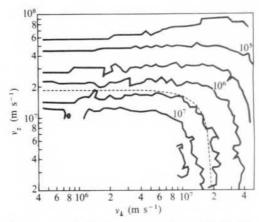


Fig. 2 The electron distribution function observed by S-310 at 09.45-09.48 UT. Phase space densities are given in units of electron cm<sup>-2</sup> s<sup>-1</sup> sr<sup>-1</sup> keV<sup>-1</sup>, and the dashed curve indicates 1 keV.

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three other detectors. All seven detectors step through 32 energy steps, and a complete distribution function such as the one shown in Fig. 2 is obtained in  $\sim$ 3 min. As each detector is sampled 11.6 times per second, the distribution shown in Fig. 2 would ideally be based on  $\sim$ 15,000 samples, but as some data contaminated by sunlight and other effects must be rejected, the real number may be a few thousands lower. The pitch angle intervals scanned by different detectors normally overlap, and this overlap is used by an intercalibration routine to compensate for differences in the efficiency of the electron multipliers.

The contours of constant level plotted in Fig. 2 are derived by dividing the measured fluxes by the particle energy and transforming the energy and pitch angle of each sample to  $(v_{\perp}, v_z)$ -coordinates. The displayed part of velocity space is then covered by a network, consisting of  $43\times35$  meshes. Between each mesh point, the logarithm of a velocity component changes by 0.05. The average value of the distribution function is calculated within each mesh, and linear interpolation between nearest neighbours is used to define the function between the mesh points. As the pitch angle resolution is limited to  $2^{\circ}$  and the logarithmic scales will expand the small pitch angle region strongly, the average flux in the intervals  $0^{\circ}$ - $2^{\circ}$  and  $2^{\circ}$ - $4^{\circ}$  pitch angle has been used. Insignificant ripple of the contours at small pitch angles can thus be avoided.

The dashed curve at 1 keV shows the shape of an isotropic distribution. The value of the distribution function is lower by a factor of  $\sim 3$  at small pitch angles than at  $\alpha = 90$ , but the positive slope in the distribution is apparently very weak.

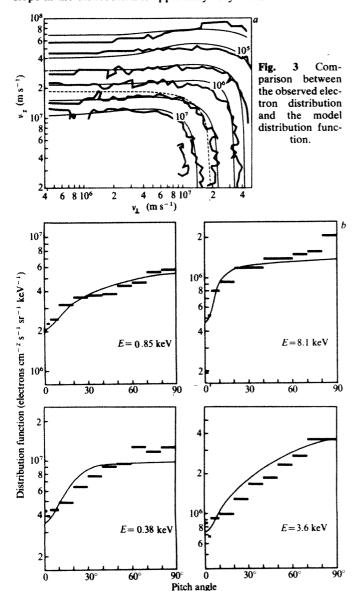


Table 1 Parameters of the complete model distribution

Component no.	Density (electron m <sup>-3</sup> )	Temperature (eV)	Δ	β
1 2	$5.5 \times 10^6$ $3.0 \times 10^6$	1 5	1	
3	$6.6 \times 10^4$	200	$\hat{0}.35$	0.15
4	$6.4 \times 10^4$	1,000	0.35	0.99
5	$5.1 \times 10^4$	8,000	0.40	0.02

A computer code can be used to estimate the growth rates of possible instabilities, if the measured distribution is modelled by a sum of components of the form<sup>12</sup>

$$f_{i} = \frac{\exp\left(-\frac{v_{z}^{2}}{V_{i}^{2}}\right)}{(\pi V_{i}^{2})^{3/2}} \left\{ \Delta_{i} \exp\left(-\frac{v_{\perp}^{2}}{V_{i}^{2}}\right) + \frac{1 - \Delta_{i}}{1 - \beta_{i}} \right.$$
$$\left. \times \left[ \exp\left(-\frac{v_{\perp}^{2}}{V_{i}^{2}}\right) - \exp\left(-\frac{v_{\perp}^{2}}{\beta_{i} V_{i}^{2}}\right) \right] \right\}$$

Here, the parameters  $\Delta_i$  and  $\beta_i$  measure the depth and width of the loss-cone, and  $V_i = (2T/m)^{1/2}$  is the thermal velocity. A model of this kind fitted to the observations is shown in Fig. 3. Figure 3a shows the contours of the model superimposed on the measured distribution. The differential fluxes as a function of  $\alpha$  are compared in Fig. 3b. It is not easy to assess the degree of agreement between model and measurement quantitatively, as the fit is done by trial and error. The model shown in Fig. 3 is close to the best obtainable using three components of the form above.

In addition to the three hot components, two maxwellian components are used to model the low-energy electrons. The parameters of the complete model distribution are given in Table 1.

The wave spectra have been calculated numerically by means of a computer code which solves the dispersion relation of waves in a magnetized plasma consisting of maxwellian components. The dielectric tensor of a maxwellian plasma can be expressed in terms of modified Bessel functions  $I_n$  and the plasma dispersion function Z (ref. 23). In the program, the dielectric tensor and its first derivatives with respect to  $\omega$ ,  $k_z$  and  $k_\perp$  are written as combinations of  $I_n$  and  $Z(\omega - n\Omega_e)/k_z$ . The full electromagnetic dispersion relation is then solved by iteration, using Newton-Raphson's method. Any two of the three variables  $\omega$ , k, and kcan be specified and solved for the third, but because k is taken to be real while  $\omega$  is complex, convergence can be ensured only when solving for  $\omega$ , and only this case will be considered here. The iteration stops when the relative error in the last estimate is  $<10^{-6}$ . We have then obtained a real frequency  $\omega_k$  and a temporal growth rate  $\gamma_k$ . However, to estimate the wave spectrum we need also the group velocity  $\mathbf{v}_{e}$ . The components of the group velocity are easily calculated from the derivatives of the dispersion relation, and the intensity amplification of the wave can be estimated as  $\gamma_k/v_g$  times the propagated distance.

When the wave propagates a distance I, the gyrofrequency will change by  $\Delta\Omega_{\rm e} \approx I \cdot \nabla \Omega_{\rm e}$ . As the unstable bandwidth is  $\sim 0.2~\Omega_{\rm e}$  (in all harmonic bands), the amplification will stop when

$$\left| \frac{\omega}{\Omega_{\rm e}} - \frac{\omega}{\Omega_{\rm e} + \Delta \Omega_{\rm e}} \right| \approx 0.2$$

In a dipole field we have  $\Delta\Omega_{\rm e}/\Omega_{\rm e} \approx 3l_{\rm r}/r_0$ , where  $l_{\rm r}$  is the distance propagated in the radial direction, and  $r_0$  is the distance from the Earth. This leads to

$$L_{\rm r} \approx \frac{0.2\Omega_{\rm e}}{3\omega} r_0$$

as the maximum radial distance over which the wave can be amplified. At  $r_0 \approx 6$   $R_{\rm E}$  and  $\omega \approx 3/2$   $\Omega_{\rm e}$ , this corresponds to  $\sim R_{\rm E}/4$ . However, the radial amplification length may not be very interesting, as the scale lengths parallel to the magnetic field and in azimuth are larger.

Experimental studies of the occurrence of these types of emission indicate that they are confined to within a few degrees of the geomagnetic equator<sup>7,10</sup>. Although the reasons for this close confinement to the equatorial plane are poorly understood, a maximum amplification length  $L_x = 2 L_t$  has been used in the calculations. This corresponds to a confinement within ~±2.5° of the geomagnetic equator. There is no way of determining the maximum amplification length  $L_{\perp}$  in azimuth from observations. As a reasonable estimate we chose  $L_{\perp} = 4 L_{r}$ . These estimates are very rough, but an accurate determination of the amplification lengths would require ray tracing in a magnetosphere where the variation of the plasma parameters in a large volume of space were known. As there is no experimental technique which measures the relevant parameters throughout a large volume, we must rely on such estimates, but note that the value of  $L_{\perp}$  chosen is compatible with the result of a simplified ray tracing in an idealized magnetospheric model<sup>12</sup>

Assuming that the wave growth is limited by propagation effects rather than some nonlinear saturation mechanism, we can calculate the shape of the frequency spectrum. At each frequency, we choose the k value which gives the strongest amplification within a region of space with dimensions  $L_{\perp}$  and  $L_{\rm r}$ . The amplification (in dB) is calculated as 8.7  $\gamma_{\rm k}$ ·min( $L_{\perp}/v_{\rm g}$ )  $L_z/v_{zz}$ ) and this amplification rate as function of  $\omega_k$  will give the approximate shape of the frequency spectrum. Absolute values of the spectral density cannot be calculated without specifying the initial level of fluctuations, but the relative strength of emissions at different frequencies can be compared with observed spectra.

The amplification predicted by the theory is compared in Fig. 4 with a measured frequency spectrum. The spectral density

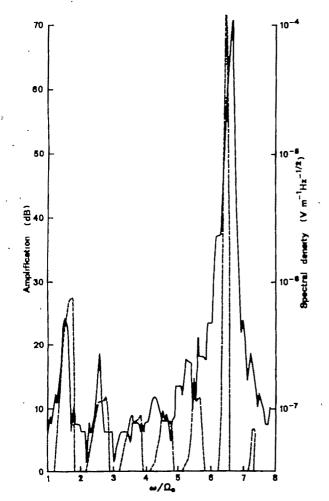


Fig. 4' Comparison between the spectral density observed by S-300 at 09 48 UT and the calculated amplification (dashed).

(solid line) was measured by the S300 experiment at around 09.48 UT on 25 August 1977. The density amplification (a dashed line) has been calculated using the model distribution function defined in Table 1. Although the exact positions of the peaks within the harmonic bands coincide poorly, the general shape of the measured spectrum is very well described by the calculated amplification. The theory correctly predicts that the strongest emission is near the upper hybrid frequency, with fairly high amplitudes around  $3/2 \Omega_{\bullet}$  and weaker signals in the intermediate harmonic bands. The upper hybrid amplitude of the very quietest emissions observed by GEOS 1 (ref. 9) is  $\sim 5 \times 10^{-6} \text{ V m}^{-1} \text{ Hz}^{-1/2}$ . Taking this value as a reasonable estimate of the background fluctuation level, we find that it is consistent with the 0 dB level chosen for the comparison in Fig. 4.

Two main types of error will affect the reliability of this comparison of theory and experiment, the most important source probably being the limited time resolution of the experiments. A measurement of the complete hot particle distribution cannot be made in <3 min, in which time all relevant parameters may vary significantly. As the low energy (0.3-1.5 keV) particles mainly involved in the instability were measured at the end of the period 09.45-09.48 UT, the spectrum chosen for the comparison has been taken near 09.48 UT to minimize the effect of time variations. However, a full frequency sweep is obtained in 22 s, and significant changes in the spectrum are often seen from one sweep to the next. This indicates that rapid variations are common, and the accuracy of the prediction is probably limited by these time variations. The second source of error is the rough estimate of the size of the amplification region. If the magnitudes of  $L_{\perp}$  and  $L_{\tau}$  are varied with the ratio  $L_{\perp}/L_{z}$  fixed, the maximum amplification at all frequencies will vary at the same rate. The amplification curve will be compressed or expanded but retain its general shape. Reasonably small changes in the ratio  $L_{\perp}/L_{\pi}$  should mainly influence the frequency of maximum amplification within each harmonic band, but small changes in the magnitude of the peaks might also occur. Compared with these two main difficulties, the errors introduced when modelling the distribution function and solving the dispersion relation are probably small.

The values of most parameters needed for this study have been determined directly from measurements, and the remaining values are at least consistent with observations. Given a set of input parameter values, it seems unlikely that the calculations would reproduce the observed wave spectrum so well if the essential physics were not included in the mathematical model. In particular, it seems possible to estimate the shape of the wave spectrum using linear theory if the particle distribution function is known. Quasilinear or nonlinear effects are apparently not required to limit the wave growth in this case, although the weakness of the gradients in the hot electron distribution may well be the result of quasilinear diffusion.

Considering the present results and those of Horne et al.20, our understanding of the linear theory of electrostatic electron cyclotron harmonic waves is probably approaching a point where quantitative predictions about wave spectra may be based solely on particle measurements. The experience of applying this methodology to a well chosen set of wave events should also enhance the possibilities of using wave measurements for magnetospheric plasma diagnostics.

This study has been performed within a large GEOS 1 collaboration and our colleagues have contributed to it in many ways. In particular we thank H. Borg, P. Gough and R. Horne for assistance in the data handling and valuable comments. This research was supported by the Swedish Board for Space Activities, the SRC and the ESA.

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### Amplitude variations of electron cyclotron harmonic waves

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Electron cyclotron harmonic (ECH) instabilities just outside the plasmapause and at frequencies near the cold upper hybrid frequency are a common feature of the Earth's magnetosphere. These waves which have virtually no magnetic component, are believed to have an important role in the generation of weak diffuse aurora<sup>1,2</sup>. They are able to interact strongly with electrons in the hundred eV to several keV energy range which can result in pitch angle scattering and precipitation on magnetic field lines which map down into the auroral zone. On the dayside magnetosphere these waves can exhibit large amplitude variations of 30-40 dB and can also exist at steady amplitudes on time scales of the order of tens of seconds. Here we seek an explanation for the sporadic nature of such instabilities by performing linear stability calculations and extending the technique used in the accompanying paper3.

An example of the emissions recorded by GEOS 1 on 25 August 1977 is shown in the frequency time spectrogram of ref. 3. Figure 1 of ref. 3 shows that after 09.45 UT in that event, the signal in the band near the upper hybrid frequency  $f_{\rm uh}$  is accompanied by lower-frequency emissions at  $\sim 3/2$  and  $\sim 5/2$  times the electron gyrofrequency  $f_{ce}$  typical of spectra of class 3 in the scheme of Gough et al.4.

In the periods 09.48-09.50, 09.51-09.52 and 09.53-09.54 UT the second harmonic of the strongest emission appears together with signals in the extra low frequency (ELF) range. The second harmonic signal is an instrumental saturation effect indicative of large wave amplitudes >3 mV m<sup>-1</sup>. The ELF signal measured in the frequency range 15-450 Hz and detected by the same preamplifier also saturates and thus preamplifier saturation effects cannot be ruled out. However, studies of similar events on GEOS 15, have shown that ELF signals detected in unsaturated conditions are electrostatic with a spectral peak close to the lower hybrid frequency. Nonlinear wavewave couplings due to the strong  $f_{\rm uh}$  pump wave are thus implied.

Time variation of  $f_{uh}$  and ELF amplitudes are shown in Fig. 1, with changes in the energy density of electrons below 500 eV.

Electrons in the energy range 0.5-500 eV are measured by the S302 particle experiment<sup>6</sup> using two detectors, one looking along the satellite spin axis and the other at 80° to it. As the angle between the spin axis and the magnetic field was 18° during this event, pitch angles between 62° and 98° were covered by the second detector.

Analysis of the data from this instrument shows, after subtraction of photoelectron contamination, that the low energy electrons can be represented by four maxwellian components at temperatures of 1, 5, 60 and 200 eV. Only the 5-eV component shows sporadic variations which are seen preferentially in the nearly field-aligned detector. Summarizing the observations in terms of an energy density, this quantity is strongly anticorrelated with the strength of waves near  $f_{\rm uh}$  and ELF emissions as shown in Fig. 1. This suggests that the wave amplitude variations might be connected with changes in the distribution of lowenergy electrons.

Higher-energy electron fluxes between 350 and 22 keV, recorded by the S310 experiment, show remarkably little variation by comparison. Data taken at intervals of 6 min, and covering times of highly variable wave activity, reveal no significant changes in the hot electron distribution. The distribution shown in Fig. 2 of ref. 3 can thus be regarded as typical for the time period of interest here.

Using the techniques described in ref. 3, two electron distributions have been constructed, one (model A) appropriate to the period of strong wave activity 09.48-09.50 UT and the other

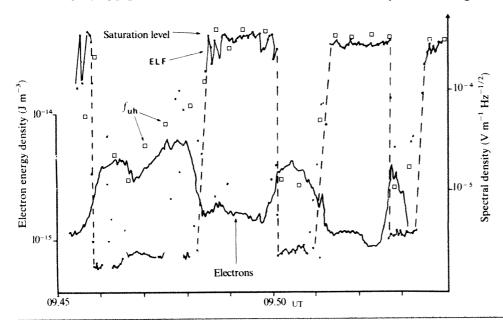


Fig. 1 Anticorrelation between the energy density of electrons below 500 eV and the amplitude of waves both in the ELF region and near the upper hybrid frequency fuh. A full pitch angle energy distribution is measured by the \$310 experiment between 09.45 and 09.48 UT. At 09.50 UT the satellite coordinates are LT = 11.20, L = 6.6, magnetic latitude = 10° and  $k_{\rm p} = 5$ .

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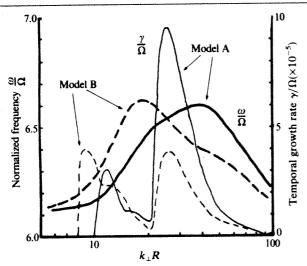


Fig. 2 Solution of the dispersion relation between the electron gyroharmonics  $6\Omega$  and  $7\Omega$ . The electron gyrofrequency is  $f_{\rm ce}=4.4$  kHz and the cold upper hybrid frequency is  $f_{\rm uh}=6.05f_{\rm ce}$ . The thick solid and dashed curves are the wave frequency for each model while the thin sharply peaked curves are the temporal growth rates. Calculated parameters include  $\alpha=52$ ,  $\Delta\omega=0.1$  for model A and  $\alpha=-22$ ,  $\Delta\omega=0.2$  for model B.

(model B) corresponding to weaker wave activity 09.45–09.48 UT. Both models use the same parameters derived in ref. 3 for the three highest energy components, while the low energy components have been remodelled from a more refined analysis of the low-energy particle data: model A consists of two isotropic maxwellians with temperatures and densities,  $T_1 = 1 \text{ eV}$ ,  $n_1 = 7.5 \times 10^6 \text{ m}^{-3}$ ,  $T_2 = 5 \text{ eV}$ ,  $n_2 = 1 \times 10^6 \text{ m}^{-3}$ . For the three components of model B the following values are used:  $T_1 = 1 \text{ eV}$ ,  $n_1 = 4.5 \times 10^6 \text{ m}^{-3}$ ;  $T_2 = 5 \text{ eV}$ ,  $n_2 = 3.5 \times 10^6 \text{ m}^{-3}$ ;  $T_3 = 10 \text{ eV}$ ,  $T_{3\perp} = 5 \text{ eV}$ ,  $n_3 = 5 \times 10^5 \text{ m}^{-3}$ . This last anisotropic component, plus the increased density of  $n_2$ , reflect reasonably well the increased fluxes at small pitch angles of suprathermal electrons which are presumably of ionospheric origin. The cold upper hybrid frequency is set at 26.6 kHz, a little lower than in previous preliminary reports.

The linear electrostatic dispersion relation for ECH wave propagation in a multi-component plasma has been solved numerically for complex frequency as a function of real wave vector components,  $k_{\perp}$ ,  $k_z$  are respectively perpendicular and parallel to the ambient magnetic field  $\mathbf{B}_o$ . The dependence of the normalized real frequency  $\omega/\Omega$  (where  $\Omega=2\pi f_{ce}$ ) is shown in Fig. 2 as a function of  $k_{\perp}R$  (R being the 200-eV electron Larmor radius of  $\sim 300\,\mathrm{m}$ ). Also plotted are the largest normalized temporal growth rates  $\gamma/\Omega$  taken for the unstable  $k_z$  R range.

A comparison of the two models shows that the enhanced 5-eV component in model B has moved the frequency maximum to smaller perpendicular wavenumbers and increased the perpendicular group velocity near  $k_{\perp}R \sim 30$ , where  $\gamma$  is large. Furthermore, increased cyclotron damping, caused by the additional 5-eV electrons, has reduced the temporal growth rate by a factor of 2 with respect to that of model A. However, the unstable waves predicted here have non-zero group velocities. They are convectively unstable and therefore the spatial growth rates must be considered to determine the final wave amplification levels<sup>10</sup>.

Spatial growth rates  $k_i$  can be calculated from the temporal growth rate and the group velocity  $(k_i = -\gamma/V_g, k_i \ll k_\perp)$ . The final amplification levels are estimated when the distance over which the amplifying waves propagate is known.

It is assumed that the plasma density variation is small over the relevant time scales and distances for convective growth.

Furthermore, changes in the dimensionless frequency  $\bar{\omega} = \frac{\omega}{\Omega}$  are assumed only to occur as the waves propagate through regions of

varying magnetic field (assumed to be dipolar). When the change in dimensionless frequency,  $\Delta \bar{\omega}$ , is such that the waves are effectively detuned from resonance, convective growth ceases and amplification is thus limited to a magnetospheric volume characterized by the three scale lengths  $L_{\rm r}$  in radius,  $L_{\perp}$  in azimuth (both in the plane perpendicular to  ${\bf B}_{\rm o}$ ) and  $L_{\rm z}$  parallel to the magnetic field. These parameters are now estimated for a dipole magnetic field.

The radial dimension  $L_r$  derived in ref. 3 is given below with the scale length  $L_{rp}$  due to plasma frequency variations assumed inversely proportional to  $R^2$  (ref. 11)

$$L_{\rm r} \simeq \frac{R_{\rm o}}{3} \frac{\Delta \bar{\omega}}{\bar{\omega}}$$
  $L_{\rm rp} \simeq \frac{R_{\rm o}}{2} \frac{\Delta \omega'}{\omega'}$ 

where  $\omega' = \omega/\omega_{\rm p}$ . In the model  $\Delta\bar{\omega}/\bar{\omega} = \Delta\omega'/\omega'$  and thus for the same percentage variation in  $\bar{\omega}$  and  $\omega'$  magnetic field variations yield the smallest scale length. Moreover calculations for different plasma densities show that the dispersion curves in the region corresponding to large temporal growth rates,  $k_{\perp}R = 30$ , are less sensitive to plasma density variations than the upper hybrid frequency is. Therefore magnetic field variations make  $L_{\rm r}$  the dominant quantity. The azimuthal dimension  $L_{\perp}$  is obtained by adopting the ray tracing method of Barbosa and Kurth<sup>12</sup> where the spatial variation of wave refractive index  $n(\bar{\omega})$  due to magnetic field changes is expressed as a power law in  $\bar{\omega}$ .

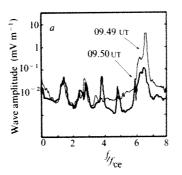
$$n(\tilde{\omega}) = \tilde{\omega}^{\alpha}$$

Here  $\alpha$  is the power index evaluated over the perpendicular wavenumber range corresponding to instability. The result is

$$L_{\perp} \approx R_{o} \left( \frac{8\Delta \tilde{\omega}}{3\tilde{\omega} (3.|\alpha| \pm 1)} \right)^{\frac{1}{2}}$$

where  $R_o=6.6$  Earth radii and the negative sign should be taken for  $\alpha>0$ . In the calculations  $\Delta\bar{\omega}$  is identified with the frequency bandwidth over which significant spatial amplification exists, with the results  $L_{\perp} \approx 600$  km for model A and  $L_{\perp} \approx 1,500$  km for model B. The parallel dimension,  $L_z$ , for this particular class of wave activity is taken from observations. Strongest signals have been reported to be confined to  $\pm 2.5^{\circ}$  about the geomagnetic equator<sup>4</sup>. More recent observations<sup>13</sup> show the confinement to be as small as  $\pm 1^{\circ}$  from which the value  $L_z=1,500$  km is used in these calculations. Note that equatorial confinement of the upper hybrid emission is probably due to a combination of refraction effects and loss of resonance in weak field gradients<sup>12</sup>.

Computations of the group velocities show that the waves traverse the amplifying region in a few tens of seconds at all frequencies except  $\sim 6.6~f_{\rm ce}$ . At this frequency, which corresponds to the maximum in the dispersion curves, the perpendicular group velocity changes sign while the parallel group velocity remains small. Predictions for model B then indicate that such a wave, which is essentially absolutely unstable, would remain in the amplifying region for some tens of minutes. That



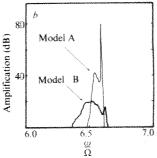


Fig. 3 Comparison between the observed wave spectra at 09.49 and 09.50 UT (a) and the calculated wave amplification between  $6\Omega$  and  $7\Omega$  (b). Strong electrostatic waves are not observed between  $7f_{ce}$  and  $8f_{ce}$  and the cold upper hybrid frequency is estimated at  $6.05 f_{ce}$  (see text).

this cannot happen can be seen from the observation that the duration of both strong and weak emissions is  $\sim 90$  s. After this period changes in the particle distribution remove the near absolute instability and increase both parallel and perpendicular group velocities.

Furthermore, detuning effects arising from wave propagation is the z direction, which are not considered in the ray tracing model above, will also occur.

The final amplification levels predicted for both models are shown in Fig. 3. Convective amplification levels have been calculated in the same way as in ref. 3 using the scale lengths deduced above. For frequencies corresponding to near absolute instability the temporal amplification is assumed to be limited to ~90 s, irrespective of whether the waves have traversed the amplifying region or not. Figure 3 also shows wave spectra representative of the two emission levels.

The prediction for model B is a spectrum peaking at the 20-dB amplification level, while that of model A exceeds it by at least 20 dB and rises to a maximum of 80 dB amplification. A significant difference is also seen in the natural wave spectrum where the maximum amplitude levels in the  $6-7f_{ce}$  band for the strong emissions (corresponding to model A) exceed that of the model B related intermediate level emissions by at least

We conclude that the model used in this study includes the main features necessary to explain the sporadic transitions between the intermediate  $(E > 100 \,\mu\text{Vm}^{-1})$  and large amplitudes  $(E > 3 \text{ mV m}^{-1})$  typical of dayside ECH emissions. These transitions are due to subtle changes in the thermal and suprathermal electron populations, and this emphasizes that detailed understanding of such emissions requires accurate information about the distribution of the suprathermal population as well as that of the 'free-energy' source. Furthermore, even if there are small changes in the free energy source, the prediction of such a large difference between the maximum model amplification levels suggests that this conclusion remains unchanged. Further calculations designed to test the sensitivity of the models to small variations in the value of the cold plasma density support these conclusions. Predictions for  $\omega_{uh}$  as low as 5.8 $\Omega$  show that the difference between the maximum amplification levels in the 6-7 $\Omega$  band is maintained, whereas for  $\omega_{uh} > 6.6\Omega$  there is virtually no difference between the levels in the  $6-7\Omega$  band but large amplitudes are also predicted above  $7\Omega$ . As strong emissions above  $7\Omega$  are not observed experimentally, we conclude that  $\omega_{uh}$  lies close to  $6\Omega$  and that the value used here of  $\omega_{\rm uh} = 6.05\Omega$  is realistic.

Finally, we point out that many observations of strong sporadic emissions have been made using the GEOS 2 satellite, and the appearance of suprathermal, approximately fieldaligned electron components, presumably of ionospheric origin, often show strong anticorrelation with the upper hybrid band emission amplitudes.

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### Crystal structure of a synthetic high silica zeolite—ZSM-39

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The crystal structure of a high silica zeolite ZSM-39, unit cell composition excluding residual water and occluded materialstetramethylammonium ion, tetraethylammonium ion)<sub>0.4</sub>(AlO<sub>2</sub>)<sub>0.4</sub>(SiO<sub>2</sub>)<sub>135.6</sub>, was determined by X-ray powder diffraction. The framework is pseudoface-centred, pseudocubic with  $a = 19.36 \pm 0.02 \text{ Å}$  and ideal symmetry Fd3m. The framework consists of a space-filling arrangement of pentagonal dodecahedra and hexakaidecahedra and is isostructural with the 17 Å cubic gas hydrate. The ZSM-39 framework is composed entirely of five- and six-rings which limits its sorptive and exchange properties. However, the large fraction of five-rings and the high Si/Al ratio (>40) impart a high thermal stability. ZSM-39 containing no aluminium constitutes the end member composition. Although ZSM-39 is the only synthetic zeolite analogue of a gas hydrate, we propose here two related hypothetical frameworks containing pentagonal dodecahedral cages.

The analogy between hydrogen bonds linking oxygens in clathrates and oxygens linking T-atoms in aluminosilicate framework structures is well known. Hexagonal ice I and ice Ic are isostructural with  $\beta$ -tridymite and  $\beta$ -cristobalite respectively<sup>1</sup>, HPF<sub>6</sub>·6H<sub>2</sub>O and (CH<sub>3</sub>)<sub>4</sub>N(OH)·5H<sub>2</sub>O are isostructural with sodalite<sup>2,3</sup> and the rare mineral, melanophlogite<sup>4-6</sup> is isostructural with the 12 Å cubic gas hydrate<sup>7,8</sup>. ZSM-39 is the first zeolite which is isostructural with the 17 Å cubic gas hydrate<sup>9,10</sup>.

ZSM-39 was synthesized hydrothermally in a system containing tetramethylammonium and tetraethylammonium hydroxide, silica, and sodium aluminate. Crystallization was at ~155 °C and was complete after 330 h (ref. 11). Analysis of a ZSM-39 sample gave (wt% composition): SiO<sub>2</sub>, 89.0%; Al<sub>2</sub>O<sub>3</sub>, 00.2%; Na<sub>2</sub>O, 00.3%; N, 00.8%; C, 03.76%. The remaining 5% could not be accounted for with complete confidence. The analytical data refer to the as-synthesized material before calcination. In this state the unspecified difference can be attributed primarily to water and hydroxyl groups.

Crystals of ZSM-39 tend to have octahedral habit as shown in Fig. 1. Single crystals > 10 μm could not be grown. ZSM-39 has

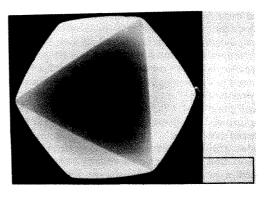


Fig. 1 Scanning electron micrograph of ZSM-39 single crystal. The habit suggests cubic symmetry. ×4,500.

Table 1	X-ray diff	raction data f	or ZSM-39	
HKL	2θ (deg)	d <sub>obs</sub> (Å)	d <sub>colc</sub> (Å)	Relative intensity
	7.50	11.79		1
None	7.93	11.15	11.18	5
111 220	12.95	6.84	6.84	23
	13.72	6.45		2
None	14.35	6.17	100000	ī
None	15.21	5.83	5.84	93
311 222	15.88	5.58	5.59	69
	16.58	5.35		1
None	18.37	4.83	4.84	47
400	20.03	4.43	4.44	36
331	21.20	4.19		2
None	22.51	3.95	3.95	48
422	23.90	3.72	3.73	100
331; 511	24.55	3.63	3.73	1
None	26.04	3.42	3.42	42
440	27.25	3.27	3.27	84
531	27.63	3.27	3.23	10
442; 600	29.16	3.06	3.06	12
620	30.26	2.95	2.95	8
533	33.05	2.71	2.71	2
551; 711	35.63	2.71	2.52	9
553; 731	35.03 37.15	2.32	2.42	í
800		2.42	2.36	10
733	38.05	2.38	2.28	17
660; 822	39.51	2.24	2.24	3
555; 751	40.34	2.24	2.22	1
662	40.64	2.22	2.16	3
840	41.70	2.17	2.10	1
753; 911	42.53	1.98	1.98	
844	45.96	1.98	1.95	3 4
771; 755; 933	46.67		1.90	2
862; 10, 20	47.91	1.90	1.86	10
666; 10, 22	48.88	1.86	1.80	6
953	50.55	1.81	1.77	
10, 42	51.72	1.77	1.69	3
955; 971; 11, 31	54.24	1.69	1.68	3
882; 10, 44	54.47	1.68	1.66	5 3 4 5 2 5
10,60	55.35	1.66	1.64	2
973; 11, 31	56.02	1.64	1.61	5
12, 00; 884	57.10	1.61		8
11, 51; 777	57.72	1.60	1.60 1.57	10
10, 64; 12, 22	58.80	1.57	1.57	5
975;11,53	59.44	1.56	1.30	3

Table 2	Atomic coordinates of	of ZSM-39 Fd3m with	origin at 3m
	x	y	Z
<i>T</i> 1	0.125	0.125	0.125
T2	0.2195	0.2195	0.2195
T3	0.1823	0.1823	0.3728
01	0.1722	0.1722	0.1722
02	0.2024	0.2024	0.2967
03	0.125	0.125	0.3743
04	0.25	0.1573	0.4073

little zeolitic sorptive capacity even after calcination at high temperatures. The material becomes black on calcination indicating that carbonaceous material is being retained in the pore structure. The same effect is observed with melanophlogite<sup>1</sup>. The thermal stability is very high and crystallinity is retained even after calcination at temperatures above 800 °C.

X-ray diffraction patterns of ZSM-39 were obtained using a Norelco powder diffractometer and  $CuK\alpha$  radiation. Powder diffraction data are given in Table 1.

Morphology, X-ray powder, and sorption data were used to determine the structure. Morphological evidence indicative of cubic symmetry was confirmed by analysis of the powder pattern and a cell parameter of  $19.36\pm0.02$  Å (D. H. Olson, personal communication) was obtained by least-squares refinement using 39 indexed reflections. Except for a few very weak lines, all reflections attributable to ZSM-39 are consistent with Fd3m symmetry. The high symmetry and lack of sorptive capacity suggested a clathrate-like structure. Examination of these structures revealed that the 17 Å cubic gas hydrate had ideal symmetry Fd3m. This structure has an average lattice constant of 17.2 Å and a hydrogen bond distance of 2.73 Å. Assuming an

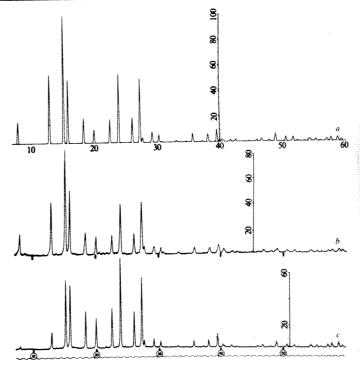


Fig. 2 a, Simulated powder pattern of the ZSM-39 framework. b, Powder pattern of ZSM-39 (sample after calcination for 0.5 h at 800 °C). c, Powder pattern of ZSM-39 (sample as synthesized).

Si—O—Si bond distance of 3.07 Å, the postulated adjusted cell parameter of the silica analogue would be 19.34 Å which compares well with the observed value of 19.36 Å for ZSM-39.

Least-squares atomic positions for the T and oxygen atoms, obtained using the DLS method<sup>12</sup> are given in Table 2. A Smith plot<sup>13</sup> obtained using these coordinates is compared with an X-ray powder pattern of ZSM-39 in Fig. 2. The agreement indicates that the ZSM-39 framework is topologically equivalent to that of the 17 Å cubic gas hydrate.

A number of very weak reflections inconsistent with a facecentred lattice indicated that the symmetry of ZSM-39 is lower than Fd3m. This is similar to the situation with melanophlogite<sup>14</sup>.

The framework of ZSM-39 consists of 12-hedra (pentagonal dodecahedra) and 16-hedra (hexakaidecahedra) as shown in Fig. 3. The structure is composed of layers of face-sharing 12-hedra arranged as in Fig. 4a. In ZSM-39 these layers are stacked in an ABC sequence. The framework may be alternately viewed as 16-hedra linked tetrahedrally through common sixrings. The arrangement of 16-hedra is the same as that of the carbon atoms in diamond.

The layers of ZSM-39 (Fig. 4a), may also be stacked in an AB sequence, giving rise to a hypothetical framework with ideal

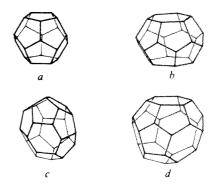


Fig. 3 Polyhedral building units of the ZSM-39 and melanophlogite structures. a, 12-hedron (dodecahedron); b, 14-hedron (tetrakaidecahedron); c, 15-hedron (pentakaidecahedron); d, 16-hedron (hexakaidecahedron).

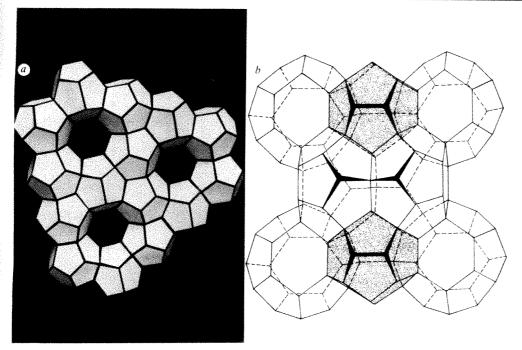


Fig. 4 a. Laver of ZSM-39 framework illustrating arrangement of face-sharing 12-hedra. b, Melanophlogite framework illustrating isolated 12-hedra.

symmetry, P6m2, and cell parameters of a = 12.2 and c =24.2 Å. This structure contains the same polyhedra as ZSM-39. These two frameworks are the simplest members of a polytypic

Melanophlogite, the first reported silica analogue of a gas hydrate, has a framework consisting of interwoven layers of 12and 14-hedra (Fig. 3). In contrast to ZSM-39, this framework contains isolated 12-hedra (Fig. 4b). Sliding alternate layers a distance of a/2 results in a hexagonal framework with ideal symmetry P6/mmm and a = 13.6 and c = 14.0 Å. This hypothetical framework consists of a space-filling arrangement of 12-, 14-, and 15-hedra (Fig. 3). Although there is no known zeolite of this type, (i-C<sub>5</sub>H<sub>11</sub>)<sub>4</sub>NF · 38H<sub>2</sub>O does adopt this structure<sup>3</sup>

Porotectosilicate analogues of water-clathrate structures which typically consist of cages comprising pentagonal rings constitute a new family of high-silica materials, most of which are still hypothetical. ZSM-39, the first synthetic member, should encourage further attempts to synthesize other members of the family including melanophlogite, a small molecule trap that has long intrigued mineralogists.

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### Hydrothermal leaching of rhyolite glass in the environment has implications for nuclear waste disposal

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Glass has been widely advocated as a suitable medium for the immobilization of high-level nuclear waste1-6. Methods of vitrification to borosilicate glass are advanced, with processes set up on a semi-industrial scale<sup>7-9</sup>, but an alternative strategy would be to incorporate waste into a high-silica glass<sup>10</sup>. It is generally proposed5.6 that vitrified radioactive waste be enclosed in metal canisters and stored underground. However, the presence of heated groundwater in rocks means that the canisters may corrode, allowing hot aqueous fluids to come into contact with the radioactive waste glass and leach out radioactive elements. Numerous laboratory tests have been performed for short periods and at relatively low temperatures to assess the leaching performances of different types of glass 11-15, however, extrapolation to predict the long-term behaviour of glasses after burial is very uncertain. I report here a microprobe analysis technique which investigated hydrothermal leaching of rhyolite glass adjacent to a fluid conduit in the Tertiary hydrothermal system of the Isle of Skye, north-west Scotland. As the composition of rhyolite and proposed high-silica radioactive waste glasses are similar, this study may help to predict the long-term leaching behaviour of such glasses after underground burial.

Fractures which formed fluid conduits for the Skye hydrothermal system have been plugged by secondary mineral growth, and now form veins which cut through the rocks. The analysed sample is cut by such a hydrothermal vein, and comes from near the centre of an acid/basic composite sill. This was exposed in 1979 in a roadstone quarry at Sconser (NG 544 318) on the Isle of Skye (Fig. 1b). The sill is intruded into the late Precambrian 'Torridonian' arkosic sandstones only 300 m from the northern margin of the Western Redhills centre of granitic

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intrusion<sup>16</sup>. A gravity survey<sup>17</sup> (Fig. 1c) suggests that the margin of the granite complex is almost vertical, and passes directly downwards into the margin of a massive basic pluton underlying Skye. The heat evolved from this body drove a huge meteoric-hydrothermal convection system, whose existence is demonstrated by very depleted <sup>18</sup>O compositions in the rocks in and around the central complex<sup>18</sup>. These  $\delta^{18}$ O compositions closely resemble those in the vicinity of the Skaergaard intrusion of Western Greenland<sup>19</sup>, suggesting that numerical modelling of the Skaergaard hydrothermal system<sup>20</sup> is applicable to Skye.

Epidote-calcite veins which cut Torridonian sandstone in Sconser quarry continue through the Tertiary sill without interruption, indicating that a single hydrothermal event affected both sill and country rocks. Therefore, the sill must have been subjected to the full duration of the hydrothermal convection system generated by the plutonic complex. Veins are predominantly aligned radially to the Redhills complex, and those which are similar in size to the studied example have a separation of ~10 cm (ref. 21). Assuming a separation of 10 cm between parallel planar fractures, comparison with Norton and Taylor's S2 numerical model of the Skaergaard suggests an integrated fluid flux of over 10,000 lper cm-fracture-length, almost all of which occurred in an initial 200,000 yr period at -400 °C. An integrated flux of this magnitude provides an effectively open system for alteration of the fracture wall rocks, ruling out the possibility that fluid composition could be buffered within the sill.

The presence of tridymite in the Coire Uaighneigh Granophyre of Skye led Brown<sup>22</sup> to deduce a value of 1.5 km for maximum post-Tertiary erosion of the Skye lava pile. As Scon-

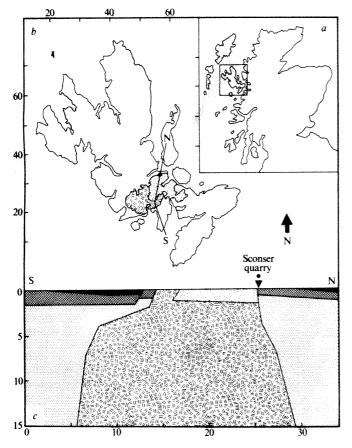


Fig. 1 a, Map of Scotland showing the location of the Isle of Skye. b, Map of Skye showing the Cuillin basic pluton (double hatching) and Redhills granites (single hatching). The spot marks the location of Sconser quarry, which lies on the line of section marked N/S. Ticks mark 10-km national grid squares. c, Crustal section through Skye based on geophysical data<sup>17</sup>. Ornament for intrusive rocks as in b. Country rocks: light stipple, Lewisian (Archaean) gneiss; heavy stipple, Torridonian sandstone; black, Mesozoic sediments. Horizontal and vertical distances in km.

ser quarry lies near sea level, this suggests that the hydrothermal vein under investigation was at a depth > 1 km, corresponding to a lithostatic pressure of 0.3 kbar or a hydrostatic pressure of 0.1 kbar. The actual pressure in the vein was probably between these two limits.

A low-power photomicrograph of the vein (Fig. 2a) shows it to be a nearly planar, sharply bounded fracture, 0.9 mm wide, filled by large sparry calcite crystals and fringed by epidote. This vein must once have been an open fissure, as euhedral prismatic epidote crystals grow out from the walls into the free space of the fracture (Fig. 2b).

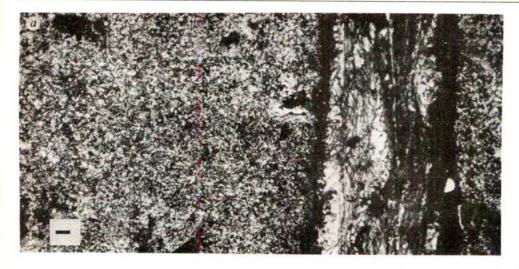
Figure 2c shows wall rock material a few mm from the vein under high power and crossed polars. Aggregates of small alkali feldspar and quartz crystals are interspersed by patches of a more isotropic matrix, showing that the microcrystalline felsitic texture of the rock was produced by devitrification of an original glass. The grainsize of the felsite falls slightly in a zone of bleached rock within 1.5 mm of the fracture. Devitrification of the original glass probably occurred during the period of hydrothermal metamorphism, rather than before or after. In this period, the presence of hot fluids would have speeded up kinetic processes of recrystallization. Figure 2d shows a map of K distribution in the felsite which picks out the distribution of alkali feldspar.

A polished section of typical wall-rock material, perpendicular to the plane of the fracture, was analysed using an electron microprobe (Cambridge Scientific Instruments Microscan 9). The probe beam of  $6\times10^{-8} A$  at  $20\,\mathrm{kV}$  was rastered over  $100\,\mu\mathrm{m}\times100\,\mu\mathrm{m}$  squares, which were analysed in 2.5 mm long strips, stretching from the vein wall into unbleached material. Twelve adjacent strips were analysed, and mean compositions were calculated for each increment of distance away from the vein. For each oxide, results outside two standard deviations from the mean were rejected, and if three or more oxides were thus excluded then the whole analysis was discounted. This procedure was adopted in an attempt to remove the effects of microphenocrysts from the felsite analyses. The area analysed was chosen for the absence of adjacent large phenocrysts.

Modal mineral analyses were determined by 'point counting' at  $40 \mu m$  intervals, using 2,500 partial microprobe analyses. The beam was focused to spot, resulting in an analysis area of a few square micrometres. The zone thus analysed was the same as for the whole-rocks.

Whole-rock microprobe analyses are plotted in Fig. 3 as profiles of oxide weight percentages against distance from the vein wall. As the vein is approached,  $SiO_2$  and MgO contents plunge, while  $Al_2O_3$ , CaO and  $Na_2O$  rise. These compositional variations extend up to a distance of  $\sim 1.5$  mm from the vein wall, beyond which major-element variations are not correlated with distance. Contents of  $K_2O$ , MnO,  $P_2O_5$ , SrO and BaO remain essentially constant from 0 to 2.5 mm from the vein, while total iron (expressed as FeO) and  $TiO_2$  vary rather erratically, due to the uneven distribution of titanomagnetite microphenocrysts (Fig. 2a).

To quantify the oxide variations as a function of distance from the vein, weight percentages have been regressed against distance, and correlation coefficients calculated. The results are shown in Table 1. Contents of SiO<sub>2</sub> and MgO display very strong positive correlations with distance from the vein, while Al<sub>2</sub>O<sub>3</sub>, CaO and Na<sub>2</sub>O display very strong negative correlations with distance. SiO2, Al2O3, CaO and Na2O form essentially linear trends between 0 and 1.5 mm from the vein, while MgO drops dramatically at 1.0 mm, before bottoming out within 0.5 mm of the vein. FeO and deficit-of-totals-below-100% (=H<sub>2</sub>O+CO<sub>2</sub>) correlate more weakly with distance from the vein, while the correlation of TiO2, K2O, SrO and BaO with distance is highly insignificant. The theoretical composition of the vein wall is calculated from the intercept of the regression lines at 0.0 mm from the vein, while the mean composition of felsite between 1.8 and 2.5 mm has also been calculated. The latter compares quite closely with the composition of 'world average rhyolite' (Table 1)<sup>23</sup>. The silica content at the vein wall (0.0 mm) is 9.34 wt% less



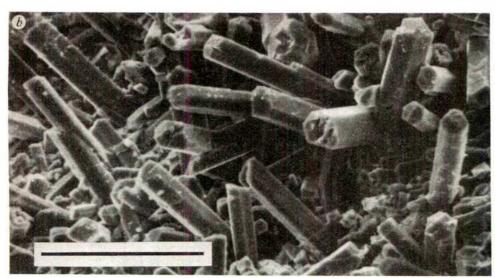
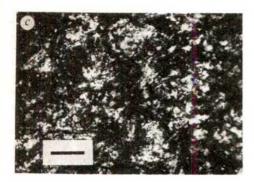
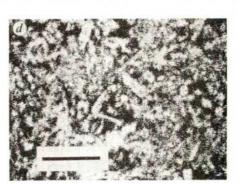


Fig. 2 a, Low-power photomicrograph of vein and analysed wall rock in plane polarized light. b, Scanning electron micrograph of prismatic epidotes growing from vein wall into fracture, after removal of vein filling calcite by dilute HCl. c, High-power view of felsite a few milimetres from vein showing devitrification texture, under crossed polars. d, X-ray backscatter picture showing K distribution in felsite 2 mm from vein.

Scale bars, 100 µm.





than in the unaltered material (1.8-2.5 mm), while the maximum enrichments of  $\text{Al}_2\text{O}_3$ , CaO and  $\text{Na}_2\text{O}$  at the vein wall sum to 9.14%. However, the build-up of these oxides cannot be a relative concentration effect caused simply by silica removal, because the enrichment factors (as a proportion of the fresh material) are different for each oxide (Table 1).

Whole-rock compositional variations in the vicinity of the vein are ascribed to exchange of species between the wall rocks and meteoric-hydrothermal fluids passing up the vein. Nevertheless, it is not immediately clear whether the changes are a product of fluid composition, or of mineralogical constraints. To assess the effects of mineralogy on whole-rock composition, modal mineral determinations were made by microprobe 'point counting'. The results are shown in Fig. 4. The abundances of calcite, epidote, quartz and alkali feldspar display correlations with distance in the region 0–1.5 mm from the vein, while beyond this distance the profiles are essentially flat. These

results are consistent with the whole-rock analyses and have similarly been regressed against distance from the vein (see Table 1).

Modal alkali feldspar content rises from an average value of 74.3% (1.8–2.5 mm from vein) to a value of 90.7% at the vein wall. Similarly, epidote increases from 0.8 to 7.5% and calcite rises from 0.5 to 1.1%. In contrast, the modal quartz content falls from 22.1% to a calculated value of -0.7% ( $\pm 1.8$ ) at the vein wall. All these elements correlate very strongly with distance from the vein (>99% confidence), but modal oxide contents do not correlate with distance at all ( $r^2 = 0.21$ ). Normative mineralogies calculated from the whole-rock compositions are consistent with modal mineral abundances. However, separation of alkali feldspar into its orthoclase and albite components shows orthoclase to be constant (31%) with distance, while albite rises sharply from 35 to 57% as the vein is approached.

Table 1 Oxide and mineral composition variations as a function of distance from the vein

	World average rhyolite	Average felsite 1.8-2.5 mm	Intercept at vein wall, 0 mm	Intercept minus 1.8-2.5 mm	Enrichment factor at vein wall	Correlation coefficient	No. of data points regressed
Oxide						. 0.00	15
SiO <sub>2</sub>	72.82	72.59	63.25	-9.34	-13	+0.98	15
$Al_2O_3$	13.27	13.50	18.45	+4.95	+37	-0.98	
TiO <sub>2</sub>	0.28	0.09	0.10	+0.01		-0.04	21
Fe <sub>2</sub> O <sub>3</sub>	1.48		AMIL.	-		-	21
FeO	1.11	1.18	0.70	-0.48	-41	+0.66	21
MnO	0.06	0.03	0.03	0	ma.	ND	
MgO	0.39	0.14	0.02	-0.12	-86	+0.98	6*
CaO	1.14	0.50	2.03	+1.53	+306	-0.84	15
Na <sub>2</sub> O	3.55	4.12	6.78	+2.66	+65	-0.98	15
K <sub>2</sub> O	4.30	5.25	5.38	+0.13		-0.16	21
$P_2O_5$	0.07	0.01	0.01	0	***	ND	
SrO	ND	0.022	0.027	+0.005	-	-0.20	15
BaO	ND	0.125	0.141	+0.016	-	-0.22	24
H <sub>2</sub> O+CO <sub>2</sub>	1.49	2.44	2.97	+0.53	+22	-0.64	20
Total	99.96	100	99.89	www.	•••	Nexi	
Mineral				.0.6	. 120	-0.71	14
Calcite		0.5	1.1	+0.6	+120		14
Oxide		2.2	1.0	-1.2		+0.21	
Epidote		0.8	7.5	+6.7	+838	-0.79	14
Quartz		22.1	-0.7	-22.8	-103	+0.92	14 14
Alkali feldspar		74.3	90.7	+16.4	+22	-0.79	14

ND, not determined.

Between 1.8 and 2.5 mm from the vein, devitrification of glass was isochemical, and formed a typical 'granitic' mineralogical assemblage of alkali feldspar and quartz with a subordinate hydrous ferromagnesian component. However, in the conditions of high water/rock ratio found at the vein wall, albite growth was particularly favoured. This developed by devitrification of glass, but also 'scavenged' additional Al2O3 and Na<sub>2</sub>O from hydrothermal fluids. In contrast, silica in the glass which was in excess of the requirements of alkali feldspar was removed by hydrothermal fluids as fast as devitrification occurred, and was not able to form quartz. The development of K feldspar seems to have been independent of water/rock ratio, as it is effectively the same at the vein wall as between 1.8 and 2.5 mm from the vein. This may also explain the flatness of the BaO profile, as the Ba<sup>2+</sup> ion is too big to fit into albite but is readily incorporated into orthoclase<sup>24</sup>. Epidote growth was strongly favoured at the vein wall, and incorporated iron, SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> and CaO from the glass, but 'scavenged' additional CaO from hydrothermal fluids. The abundance of CaO in these fluids is indicated by the plugging of the vein with calcite, which does not form a major component in the altered wall rock.

McCarthy et al.25 noted that most leachability tests on borosilicate glass are conducted at 25 °C and 1 atm, whereas temperatures<sup>26</sup> in underground waste repositories could reach 400 °C. In view of these dangers, the proposed radioactive waste content of glasses has been reduced from its original 25% (ref. 11), but the cores of full size 'production' waste blocks will almost certainly exceed temperatures of 100 °C for long periods of time. In these conditions it cannot be assumed that the metal canister enclosing a waste block will not be breached by corrosion. Observed fracture development in such blocks yields glass lumps of average radius 5 cm (ref. 27), and ensures that if such a breach does occur then fluids can penetrate to the high-temperature core of the waste block.

A leaching experiment on 11-yr old 'Fingal' radioactive waste glass at 90 °C and 1 bar revealed 0.0023 g cm<sup>-2</sup> of leaching in 1 week, yielding an estimated linear leaching rate of 40 cm in 1,000 yr (ref. 11). This would be sufficient to disintegrate a waste block fractured into 5-cm radius pieces in <100 yr. Similarly, McCarthy et al. 25 found that 4-mm spheroids of simulated-waste borosilicate glass broke into fragments and 'seemed to be totally

altered' after only 2 weeks at  $\sim 300$  °C and 300 bar. In contrast, integration of the degree of oxide depletion in the rock analysed in this work, between the vein wall and a distance of 1.5 mm away, indicates that the total quantity of material leached by fluids per cm2 of vein wall was 0.019 g. This occurred in the presence of a high fluid flux at 400 °C and 300 bar, which lasted perhaps 200,000 yr. These comparisons show that high-silica glass has a much higher resistance to high temperature hydrothermal leaching than borosilicate glass.

Karkhanis et al.15 found that disks of rhyolite glass leached with heated distilled water produced large amounts of musco vite, whereas those reacted in the presence of granite shor only a small amount of crystalline product which appeare feldspar. This observation is consistent with the presof alteration processes; formation of the stable spar/epidote alteration assemblage being my 'scavenging' of Ca2+, Na+ and Al3+ from hyd Sconser, the hydrothermal fluids must species by interaction with the ark country rocks. These sediments he granite eutectic28, suggesting the favourable geochemical env radioactive waste glass. It is important to as

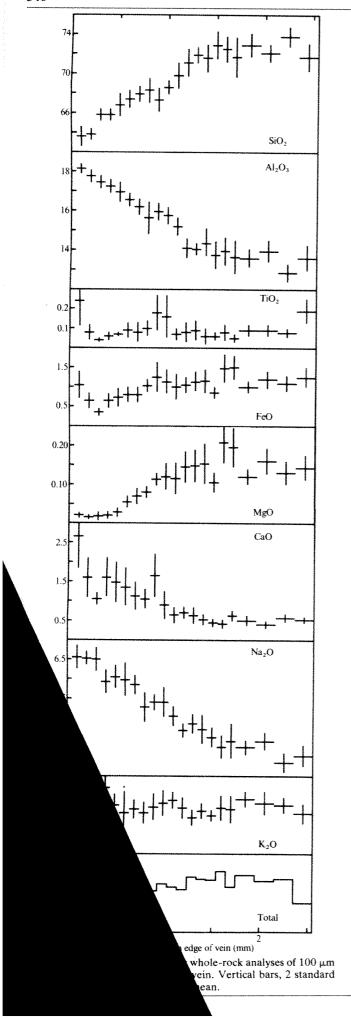
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<sup>\*</sup> Not starting at vein wall.



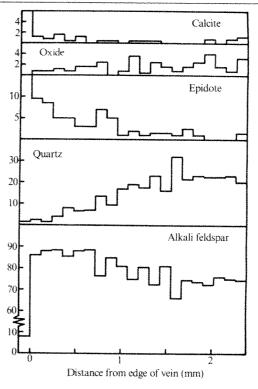


Fig. 4 Modal mineral percentages as a function of distance from vein.

slight enrichment of SrO as the vein is approached here (Table 1) shows that feldspar successfully incorporated Sr released by glass devitrification. Growth of potash feldspar enabled similar incorporation of the heavy fission product barium (Table 1). In the most severely altered material near the vein, epidote is a major hydrothermal mineral phase. Studies of hydrothermal borehole mineralogy<sup>30</sup> indicate that conditions for epidote growth have been achieved in the natural environment at depths of only 450 m. Analyses of the epidote group mineral allanite, which has grown in hydrothermal conditions in Skye31, reveal massive concentrations of actinides, rare-earth elements and strontium. The large A sites in this mineral can readily accommodate and trap LIL radioactive elements released during glass devitrification. Thus a new stable hydrothermal mineralogy can develop which will incorporate radioactive elements. However, glass must retain its structural integrity long enough for this hydrothermal mineralogy to develop.

This reveals the deficiency of borosilicate glass; the solubility of the borate fraction is such that it is readily removed from the system, causing the glass to disintegrate, and the radioactive elements to be released uncontrollably. Hence a lower borate fraction in radioactive waste glass will clearly result in greater resistance to leaching. Because borates form a flux for the melting process, their removal will necessitate higher furnace temperatures. However, if a colloidal solution of nitrates, with components present in similar porportions to the rhyolite eutectic, is evaporated, then dry melting should be possible at little over 1,000 °C (ref. 32).

That rhyolite glass retains its structural integrity and displays low solubility rates even when subject to devitrification and prolonged high temperature hydrothermal leaching indicates that high-silica glass is more suitable for long term disposal of radioactive wastes than borosilicate glass. Although high-silica glass requires a greater furnace temperature for melting, the extra cost involved may be outweighed by its stability advantages over borosilicate glass.

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#### Fission track dating of zeolites

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The fission track method has become acceptable in geochronology, not only in straightforward dating of igneous and metamorphic events but also in stratigraphic studies by the dating of ash layers1,2, the elucidation of crustal uplift history3and in archaeology8. Such studies have mostly used accessory minerals such as apatite, sphene and zircon, whose track-retentive properties are well documented. However, fission tracks can be developed and observed under the optical microscope in most minerals<sup>9-11</sup>. We report here on reconnaissance studies of fission track dating of zeolites, a complex group of tectosilicates, which is, for example, widely developed in basaltic rocks after their emplacement by later stage processes. We describe the etching conditions for some zeolites, in particular the minerals chabazite, stilbite and heulandite12 and detail the track recording and retention characteristics for chabazite, with particular reference to fossil hydrothermal systems in the Faeroe Islands.

Fleischer et al. 13 have previously reported the general etching conditions for zeolites. We have particularly investigated the effect of NaOH and KOH on chabazites. Homogeneous, inclusion-free chabazite crystals were annealed at 540 °C for 2 h

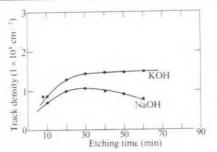


Fig. 1 Etching rates of chabazite for NaOH and KOH.

to erase all spontaneous tracks, mounted in epoxy resin and mechanically polished. The chabazite crystal structure only breaks down at 840 °C (ref. 14). Artificial tracks were then created using a  $^{252}$ Cf source in a  $2\pi$  geometry and the chemical etching was carried out in two groups for various times using boiling NaOH and KOH. The results (Fig. 1) indicate that with NaOH a maximum is reached, followed by a decrease in track density due to the progressive removal of surface layers of changed crystal. In the case of KOH (some typical etch pits are shown in Fig. 2) a plateau, reached after a relatively short time, remains constant. These relationships are due to differences in the velocity of etching along the tracks  $(V_t)$  relative to that for the bulk solid  $(V_g)$ . The ratio  $V_t/V_g$  is high for KOH but is much lower for NaOH. Note that uranium in chabazite is generally not considered to be an exchangeable ion.

Because thermal events may affect the final fission track 'age' of a sample we have carried out experiments both to evaluate this effect and to provide a calibration curve: chabazite is used as an example. If samples containing fission damage trails are subject to some thermal event and thereby lose a certain fraction of the tracks, they will appear to have decreased ages. Strozer and Wagner<sup>15</sup> proposed a method of correction for thermally lowered track densities, using calibration curves of residual track density as a function of average length of etch pit, both quantities being expressed as fractions of their unannealed values 16-18. After annealing a chabazite at 520 °C for 6 h induced tracks were created at a thermal neutron dose of 1015 (nvt). The fragment track density was then determined by etching aliquots which had undergone heat treatments varying from 100 to 500 °C (±5 °C) for 1 h. Etching through cleavage [1011] and [1101] planes was used to reveal the full track lengths. The length was evaluated by measuring both track dip and projected length, and phase contrast methods were used to enhance precision. An average of 'major' and 'minor' axes for each etch pit was recorded as the corresponding 'etch pit length'. For almost 11,000 etch pits scanned, between 800 and 900 track lengths were measured. Figure 3a shows the relationship between reduction in track density and length as a function of

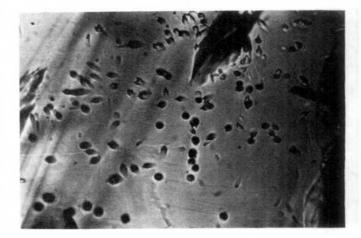


Fig. 2 Spontaneous fission tracks in chabazite etched in KOH (3 g in 4 g H<sub>2</sub>O).

		Table 1 Fission track	results of minerals fr	om the Faeroe Islar	nds		
No.	Sample location*	ρ <sub>s</sub> (cm <sup>-2</sup> )	ρ <sub>i</sub> (cm <sup>-2</sup> )	Dose (n)	N	r	Age $(Myr \pm 1\sigma)$
Cha	ibazite						
1	Runavik (US) Eysturoy	$1.58 \times 10^4$ (464)	$1.72 \times 10^5$ (625)	$7.52 \times 10^{15}$	4	0.995	$42.5 \pm 1.0$
1‡	Runavik	$1.58 \times 10^4$	$1.35 \times 10^5$	$5.84 \times 10^{15}$	6	0.989	$42.1 \pm 1.7$
2	Eysturoy (US) Slaettafjall	$(810) \\ 1.70 \times 10^4$	$(2,071)$ $3.44 \times 10^4$	$1.51 \times 10^{15}$	6	0.959	$45.9 \pm 1.0$
3	Eysturoy (US) Malinsfjall	$1,436$ $1.52 \times 10^3$	$2,309$ $1.34 \times 10^4$	$5.84 \times 10^{15}$	5	0.954	$40.8 \pm 1.3$
3+	Vidoy (US) Malinsfjall	$(430)$ $1.51 \times 10^3$	$(1,295)$ $2.19 \times 10^4$	$9.80 \times 10^{15}$	5	0.938	41.6±1.1
	Vidoy (US)	550	1,667		-		
4	Nordara Sandoy (US)	$1.75 \times 10^4$ $1,102$	$3.79 \times 10^4$ $1,696$	$1.51\times19^{15}$	6	0.965	$42.9 \pm 1.0$
5	Eidi Eysturoy (MS)	$7.14 \times 10^4$ $1.421$	$1.30 \times 10^5$ $2,286$	$1.42 \times 10^{15}$	6	0.958	$48.0 \pm 1.0$
6	Tvöroyri Suduroy (LS)	$6.08 \times 10^4$ $179$	$3.05 \times 10^{5}$ $1,565$	$4.23 \times 10^{15}$	5	0.679	$51.9 \pm 2.0$
Still	•		*,5 00				
	Runavik	$6.30 \times 10^4$ $350$	$1.28 \times 10^{5}$ $2,488$	$1.51 \times 10^{15}$	7	0.989	$45.7 \pm 1.2$
8	Eysturoy (US) Gassá-Breida	$1.63 \times 10^4$	$5.43 \times 10^4$	$2.48 \times 10^{15}$	8	0.934	$45.8 \pm 0.9$
9	Streymoy (US) Sörvagur	$883 \\ 2.87 \times 10^4$	$1,565$ $2.44 \times 10^{5}$	$6.24 \times 10^{15}$	6	0.754	$45.2\pm1.7$
9‡	Vagar† (MS) Sörvagur	$2,150$ $2.87 \times 10^4$	$2,200$ $2.77 \times 10^{5}$	$7.12 \times 10^{15}$	8	0.943	$45.4 \pm 1.9$
10	Vagar (MS) Harnar	$(234)$ $4.35 \times 10^4$	$(1,112)$ $1.26 \times 10^5$	$2.49 \times 10^{15}$	6	0.931	$52.9 \pm 2.1$
10‡	Suduroy (LS) Harnar	$367$ $4.36 \times 10^4$	$1,384$ $3.63 \times 10^5$	$7.12 \times 10^{15}$	6	0.892	$52.6 \pm 2.2$
11	Suduroy (LS) Frodböur	$(201)$ $4.56 \times 10^{5}$	$(519)$ $7.65 \times 10^{5}$	$1.51 \times 10^{15}$	8	0.768	$55.4 \pm 2.5$
	Suduroy (LS)	240	533				
Hei 12	slandite Smorbushellisgjogv Mykines (LS)	$4.96 \times 10^4$ 189	$4.52 \times 10^5$ 1113	$8.04 \times 10^{15}$	8	0.973	$54.3 \pm 2.0$

 $\rho_s$  is the spontaneous track density;  $\rho_i$  the induced track density; n, the thermal neutron dose (nvt); N the number of grains counted; R the correlation coefficient.

temperature. This information gives an immediate estimate of the degree of fading of the spontaneous tracks and hence the age reduction provided that the track lengths are measured. Chabazite has a measured average track length of 11.8± 2.1 µm. At 100 °C, a slight decrease of ~2.8% in the track density and ~3.5% in the mean track length of etch pits was observed. At higher temperatures, the track length decreased more rapidly than the track density. After 1 h at 500 °C constant temperature the track density was reduced by 57.3% and the track length by 78.3% After 1 h at 600 °C all the tracks were annealed out. The reduction in track density was observed to lag behind that of track length (Fig. 3a). U<sup>235</sup>-induced fission tracks in both unannealed and annealed samples were measured. The shrinkage in track length, plotted as a function of track density reduction, yields the appropriate calibration relationship. The ratio of mean etch pit length of annealed and unannealed fission tracks is shown as a function of the percentage of fission track density reduction in Fig. 3b. The resulting calibration curve has been used to correct thermally-lowered fission track ages of chabazite (estimated correction is ~3%). Studies of various etchants for stilbite and heulandite resulted in the use of 2% HF at 23 °C for 20-30 s and 10 ml aqua regia: 1 ml 2% HF at 23 °C for 30-50 s respectively for these two minerals.

The zeolite specimens were dated by the (EDM) detector method<sup>19</sup>, samples of chabazite, stilbite and heulandite being sandwiched between muscovite mica detectors for measuring the induced tracks. The integrated thermal neutron dose to which the samples were exposed was itself determined by irradiating calibrated glass dosimeters with each consignment of

zeolites. Each glass dosimeter (supplied by R. Fleischer) was fractured and the fresh surfaces were etched with newly prepared 20% HF for ~30–35 s at 23 °C to obtain well defined, easily distinguished etch pits, ~800–1,000 pits being scanned for each consignment. The surface density of etch pits was measured and the integrated neutron flux calculated using the calibration relation<sup>20,21</sup>, and a factor of  $2.26 \times 10^{11}$  neutrons per track. Sample numbers 1, 3, 9 and 10 were again dated where muscovite detector was used for neutron dose measurement. Thermal neutron dose was determined by counting tracks in muscovite detector irradiated in contact with standard NBS glasses SRM-962 and SRM-963 (Cu values)<sup>22</sup>; the values obtained agreed with the independent measurements made on Au foil. The analytical results are in accord with the age results given in Table 1.

An investigation of the general relationship between track counting on internal mineral surfaces and that on external detectors was also made to determine the geometric factor  $^{23}$  for the use of external detectors with zeolites. For the zeolites studied the geometric factor varied between  $0.51\pm0.02$  and  $0.57\pm0.03$ , assuming the etching rate and the counting efficiency for external detectors to be the same for each zeolite, and the track shape to be virtually the same. However, etching of tracks lying in different orientations was highly anisotropic, giving rise to etch pits of varying topographic appearance. The constants used in the age calculations were  $\lambda_f = 6.8 \times 10^{-17} \text{ yr}^{-1}$ ,  $I = 7.223 \times 10^{-3}$ ,  $\sigma = 5.802 \times 10^{-22} \text{ cm}^2$  and  $\lambda_d = 1.551 \times 10^{-10} \text{ yr}^{-1}$ . Errors were calculated according to the method of McGee and Johnson<sup>24</sup> and Naeser *et al.*<sup>25</sup>, expressed in 1 s.d.

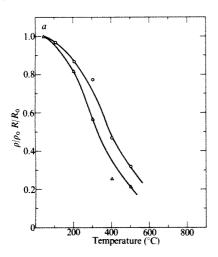
<sup>\*</sup> US, Upper series; MS, middle series; LS, lower series.

<sup>†</sup> Fissure-filling, all others are from amygdales.

<sup>‡</sup> Muscovite detector used for neutron dose determination. (For further details see text.)

The exposed part of the Faeroe Islands consists of up to 3 km of largely basaltic lavas divided into a lower, middle and upper series by stratigraphic marker horizons. There are distinct mineralogical and geochemical differences throughout the pile (see refs 26-29). The lithologies show that significant time gaps separated the three series, but this has not shown up in radiometric dating, where variable ages between  $49.2 \pm 1.5$ - $60.4 \pm 1.4$  Myr were obtained<sup>30</sup>. Subsequent refinement of these data<sup>31</sup> suggests that the Faeroese lava pile from the bottom of the lower to the top of the middle series was erupted in the interval  $55.2 \pm 1.0 - 54.6 \pm 1.2$  Myr ago.

Fission track results for chabazite, stilbite and heulandite are shown in Table 1 for samples shown in Fig. 4 where a spread of ages from  $40.8\pm1.3$  to  $55.4\pm2.5$  Myr have been found. The oldest values occur in the lower series and the youngest in the upper series. However, we have no other evidence to contradict the K-Ar results which indicate a relatively short time span for the volcanic activity, consistent with results from the contemporaneous East Greenland and lavas<sup>32</sup>. The observed distribution is probably regional, with the youngest ages in the northeastern part of the islands, reflecting a more prolonged cooling in that region. Ages from the lower series on Suduroy and Mykines, which are close to the accepted age of volcanism, indicate that in the south and west of the islands the lavas underwent rapid cooling either as a result of lack of burial by significant amounts of overlying material, or as a result of a very rapid uplift. Such rapid uplift is probably due to the domeshaped structures believed to be present offshore to the northwest of Mykines and under Suduroy<sup>33</sup>. These domes, although occurring on a much smaller scale, invite comparison with East



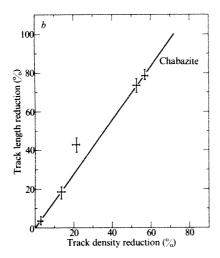


Fig. 3  $a, R/R_0$  ( $\triangle$ ) and  $\rho/\rho_0$  ( $\bigcirc$ ) as a function of temperature Tfor chabazite. b, Percentage reduction of fission track density versus track length in chabazite.

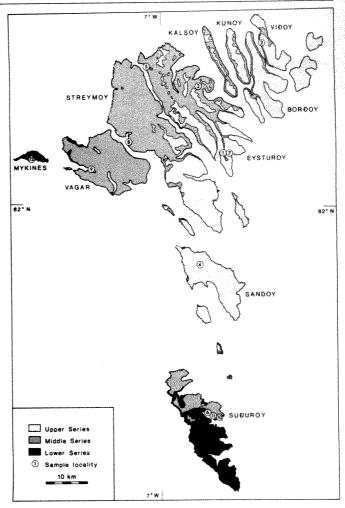


Fig. 4 Geological map of the Faeroe Islands after ref. 26.

Greenland, where a large domal uplift<sup>34</sup> was raised shortly after the basaltic volcanism and rapidly dissected by erosion4.

The annealing experiments reveal that the thermal stability of tracks in chabazite is lower than that in sphenes, garnet, epidote, allanite and hornblende<sup>35</sup>. Data from both annealing experiments and track shrinkage suggest that ages determined by applying the fission track method to zeolites will be slightly affected by thermal annealing. We assume in fact that the tracks became stable rather suddenly. Nevertheless, our study certainly indicates that the fission track method is suitable for zeolite dating. Work on the Faeroese zeolites will continue to try to clarify the detailed history of the lava pile.

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#### Comb-layering in carbonatite dykes

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Carbonatitic dykes from the Tertiary Kaiserstuhl volcano in the Upper Rhinegraben, FRG, commonly show fine-banded layering with skeletal branching calcite crystals oriented perpendicular to the layering. These calcite comb-layers are produced from magmatic in-situ crystallization in specific conditions of supercooling and rapid cooling rates 1,2. We show here that the crystallization features underline the magmatic origin of the observed textures, mineral phases and melt compositions and are a key to the physicochemical nature of the carbonatitic magma.

The term 'comb-layering' has been applied to textures in magmatic rocks characterized by layers of elongate, parallel and branching crystals with preferential orientation perpendicular to the plane of layering3. It is mostly feldspars, pyroxenes, olivine and amphibole that form the elongate crystals of comb-layers in magmatic intrusions. Moore4 found comb-layers of perovskite in olivine-melilitite and Dawson (unpublished data) observed sphene comb-layers in an ijolithe block from Oldoinyo Lengai. One example of elongate and branching calcite has been reported from a kimberlite sill at Benfontein, South Africa5. Calcite dendrites have been found in some natural carbonatites<sup>6,7</sup> and in calcite melting experiments<sup>8,9</sup>.

In general, natural examples of comb-layering have been explained as the result of crystallization from a supercooled magmatic melt. Crystallization experiments have reproduced these comb-layer textures2 and confirmed that specific conditions of supercooling, supersaturation and cooling rates are responsible for their formation.

Carbonatites in the Tertiary Kaiserstuhl alkaline complex occur as three different rock types<sup>10-12</sup>: (1) a central intrusion of coarse-grained sövite; (2) several hundred dykes cutting the subvolcanic and volcanic silicate rocks; and (3) carbonatitic lapillistones and ashes formed by the surface eruption of the carbonatite magma. The carbonatitic dykes (<1 cm to 1 m wide) exhibit a wide variety of textures from trachytoid and skeletal to porphyritic and granular. Most of the dykes consist of >90 vol.% of calcite; only a few contain larger amounts of silicate phases. Magnetite, apatite and phlogopitic mica are the main non-carbonate minerals. All dykes are alvikitic according to the nomenclature of Le Bas<sup>13</sup>.

Comb-layering parallel to the dyke margins has been observed in many of these dykes. The arrangement of layers is very regular, with a well-defined median symmetry plane and pairs of corresponding layers on either side of the centre (Figs 1, 2). The layers consist of elongate calcite crystals with a strictly parallel orientation perpendicular to the layering. Narrow dykes (maximum width 10 cm) may consist of one pair of symmetrical comb-layers only ('simply-layered dykes') (Fig. 1). Wider dykes

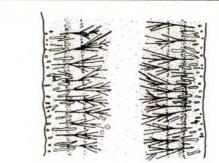






Fig. 1 a, Cross-section through simply-layered carbonatite dyke (width 5cm) with one pair of symmetrical comb-layers formed by skeletal calcite dendrites. Border vesicle zones and a fine-grained central zone are also developed. b, Micrograph of cross-section of a simply-layered carbonatite dyke with a comb-layer of columnar calcite and a granular central 'pipe' zone.

sometimes show a rhythmic banding with a succession of several comb-layers, for example Fig. 2. The layers are separated by thin bands of fine-grained carbonatite. A zone of aphyric, very fine-grained carbonatite which separates comb-layers from the actual wall-rock contact is interpreted as the chilled margin.

Single comb-layers can be up to 3 cm wide or considerably smaller (<1 mm). The elongate calcite crystals reach a length of ~2 cm with a maximum length-to-width ratio of 150:1. The elongation is perpendicular to the c-axis. Mostly the crystals are skeletal and show dendritic bifurcations (Fig. 3). The dendrites always branch towards the centre of the dykes where the elongate comb-layer calcites become closely spaced forming fanshaped bundles. A curvature of single dendritic and skeletal calcite crystals is common (Fig. 3a). The interstitial matrix between the skeletal crystals consists of granular or trachytoid calcite with extremely fine-grained (<30 µm) acicular apatite

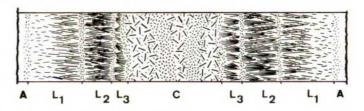


Fig. 2 Schematic cross-section through rhythmically layered comb-layer carbonatite dyke (width 25cm) A, aphyric border; L<sub>1-3</sub>, symmetrical pairs of comb-layers; C, microporphyritic to equigranular central zone.

and euhedral magnetite in varying amounts. Sometimes tiny

pyrochlore octahedra may be present.

The mineral chemistry<sup>12</sup> of calcite is rather uniform with very little variation between different dykes or single layers in one dyke. It is pure calcite with ∑MnCO<sub>3</sub>+FeCO<sub>3</sub>+MgCO<sub>3</sub>< 0.5 wt%. The SrCO<sub>3</sub> content is high and averages to 2 wt%. Apatite chemistry is characterized by high rare earth element contents (average La<sub>2</sub>O<sub>3</sub> 0.5, Ce<sub>2</sub>O<sub>3</sub> 1.0 wt%) and significant SiO<sub>2</sub> contents (up to 3.0 wt%). Low flourine concentrations (average 0.75 wt%) are in marked contrast to higher F values in apatites of the Kaiserstuhl sövites (average 2 wt%).

The granular central part between the comb-layers varies in thickness from a few millimetres in simply-layered dykes (Fig. 1) to several centimetres (Fig. 2). The contact between the centre and the comb-layer is often rather sharp. Some of the larger central zones show flow structures and in a few cases contain detached pieces of adjacent comb-layers. Vesicle zones parallel to the border occur in a few dykes. The vesicles may be void but are often filled with pure calcitic material. In the comb-layers these vesicles are elongate parallel to the dendrites. In some dykes they show a striking similarity to the carbonatitic 'migra-

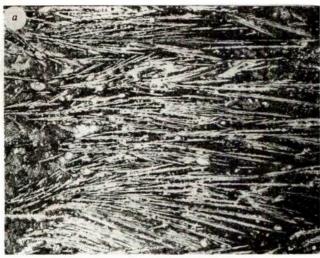




Fig. 3 a, Skeletal and dendritic calcite crystals with a slight curvature forming comb-layer of a carbonatite dyke (photographed area: 8.5×6.5 mm). b, Small bundles of calcite dendrites branching towards the centre of the carbonatite dyke. The dark matrix between the dendrites is rich in extremely fine-grained magnetite and apatite (photographed area: 3.25 × 2.5 mm).

tion vesicles' described by Dawson and Hawthorne5 from the kimberlite sill at Benfontein. In two dykes apatite comb-layers occur as well as the calcite ones. The apatite crystals are elongate and skeletal and branch towards the dyke centre.

Simply and rhythmically layered dykes have been chemically analysed (Table 1). Their composition shows the characteristic trace-element enrichment (for example, Sr, Ba, Nb, light rareearth elements), which is typically carbonatitic and compares well with other carbonatitic rocks of the Kaiserstuhl. There is a close chemical similarity with rapidly quenched volcanic lapilli which occur in the form of carbonatite lava droplets in the Kaiserstuhl (Table 1, no. 5). With H. W. Hubberten (unpublished data) we have studied carbon- and oxygen- isotope ratios of carbonatites from the Kaiserstuhl and analysed several comblayer zones. Their isotope ratios are within the narrow limits of primary carbonatites <sup>14,15</sup>. Values as low as -5 to -7% for  $\delta$  <sup>13</sup>C (PDB) and +5 to +8% for δ18O (SMOW) have been determined.

The conditions necessary for comb-layer formation have been discussed by Donaldson2, briefly they are: a compositional or thermal gradient causing unidirectional solidification; a degree of supercooling at which homogeneous nucleation in the melt is low and crystal growth is fast; a cooling rate that allows isotherms to advance at the same rate as the elongate crystal growth rate.

The almost monomineralic nature of the carbonatite melt reduces the importance of compositional factors on the constrained crystal growth of calcite. In this respect carbonatite magmas more closely resemble metal melts than multicomponent silicate magmas. The small, simply-layered carbonatite dykes therefore may be regarded as natural analogues of metal casts with 'columnar' wall-zones and a central 'pipe' zone (Fig. 1b). The main reason, therefore, for directed crystal growth in the narrow carbonatite dykes is supercooling in a high thermal gradient perpendicular to the dyke walls. The presence of comb-layering with elongate calcite in very narrow dykes (<1 cm) shows a high growth rate of calcite at rapid cooling. If the cooling rate exceeds the growth rate, supercooling in the

Table 1 Chemical composition of comb-layered carbonatite dykes. Comparison with other carbonatite rocks from the Kaiserstuhl shows the typical chemical features of carbonatites

	1	2	3	4	5
SiO <sub>2</sub>	2.22	1.74	1.69	1.37	1.02
TiO <sub>2</sub>	0.10	0.08	0.09	0.05	0.05
$Al_2O_3$	0.58	0.66	0.48	0.25	0.36
Fe <sub>2</sub> O <sub>3tot</sub>	3.03	3.97	3.03	3.18	1.67
MnO	0.46	0.57	0.69	0.42	0.54
MgO	0.45	1.15	0.73	3.05	0.45
CaO	49.2	48.7	49.0	48.6	51.7
Na <sub>2</sub> O	0.10	0.00	0.08	0.10	0.05
K <sub>2</sub> O	0.05	0.00	0.09	0.03	0.02
P2O5	1.87	2.45	1.74	2.87	1.39
LOI	38.7	37.3	38.4	37.2	40.5
V	230	150	240	90	110
Sr	11,300	7,700	7,850	9,000	6,000
Y	100	100	60	75	25
Zr	45	140	60	180	15
Nb	1,030	1,020	\$10	2,400	670
Ba	1,220	740	1,800	650	1,200
La	1,000	910	640	450	350
Ce	1,850	1,770	1,100	1,100	560
Nd	400	440	230	260	110

Analyses were by X-ray fluorescence.

Column 1: Simply-layered carbonatite dyke, 3cm wide; Oberbergen (bulk sample).

layered carbonatite dyke, 25cm wide; Column 2: Rhythmically Schelingen. Bulk sample, single layers show no significant variation.

Column 3: Average of 51 carbonatite dykes from the Kaiserstuhl.

Column 4: Coarse-grained sövite; Schelingen.

Column 5: Carbonatite lapilli; Kirchberg, Niederrotweil.

magma ahead of the solidification front will soon reach a value at which homogeneous nucleation takes place and comb-layer formation stops<sup>2</sup>. We conclude that comb-layered carbonatite dykes originated by intrusion of an almost nuclei-free melt in which heterogeneous nucleation only occurred at the dyke walls. A specific degree of supercooling maintained the low rate of homogeneous nucleation and elongate calcite crystals could grow along a thermal gradient in a rapidly cooling dyke. Experimental results 1 suggest that higher degrees of supercooling are required for comb-layer formation in melts of lower viscosity. Intrusive features of dykes and the nature of extrusive carbonatites of identical composition 11 suggest an extremely low viscosity of the carbonatite magma.

The origin of rhythmically layered carbonatite dykes is more complex. Compositional supersaturation can explain alternating comb-layers of differing mineralogy<sup>1</sup> and is suggested for the origin of the rare succession of calcite and apatite layers. Compositional supersaturation cannot be evoked for the sequence of comb-layers of very similar composition such as the calcite layers in carbonatite dykes. Periodic buildup and release of volatile pressure have been suggested as a cause for rhythmic banding<sup>1,2</sup>. This seems plausible for the shallow subvolcanic to near-surface environment in which the dykes crystallized. As comb-layer crystallization proceeds the melt becomes enriched in volatiles. This causes depression of the liquidus and stops the growth of elongate and skeletal calcite. A boundary layer is formed along which a new comb-layer starts crystallizing when appropriate supercooling conditions are re-established.

The above interpretation of banded comb-layered carbonatite dykes gives important data on the nature of the carbonatite magma. The intruding melt was almost completely liquid and dendritic calcite crystals are evidence of primary textures. Calcite was the liquidus phase in the carbonatite magma. Secondary recrystallization is of very minor importance in the present examples. The rapid cooling in narrow dykes suggests that the composition of analysed carbonatites is a good approximation to the original composition of carbonatitic melt.

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#### **Biologically damaging radiation** amplified by ozone depletions

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Many recent studies 1-3 indicate that releases of chlorofluorocarbons (CFCs)—mainly chlorofluoromethanes (CFMs)—into the atmosphere deplete the stratospheric ozone layer. Potentially dangerous consequences of these ozone depletions, such as increases in skin cancer, are expected due to a subsequent

increase in biologically damaging solar UV radiation reaching the ground. The biological effectiveness of this radiation amplification can be quantified by a radiation amplification factor (RAF) for which a value of 2 has been previously assumed: RAF = 2 means, for example, that a 1% ozone depletion will result in a 2% increase in damaging UV dose at ground level. Using accurate radiative transfer calculations together with a detailed modern data base, we calculate here the RAFs for erythemally and DNA-weighted UV-B dose assuming ozone depletions as resulting from a two-dimensional model, as well as a global ozone depletion of 10%. The amplification factor as a function of latitude and season is found to be between 1.9 and 2.2 for erythema and between 2.5 and 2.8 for DNA. This is a smaller range variation than previously claimed.

In theoretical investigations of the problem using onedimensional numerical models, the most probable value calculated for the eventual steady-state ozone depletion due to continued release of F-11 and F-12 at the 1977 level is 5-10%. New values of some of the chemical rate coefficients, such as the reaction rate of OH with HNO<sub>3</sub> (ref. 4), lead to this ozone depletion. Studies using two-dimensional models indicate that any change in ozone will not be uniform, but will show variations with latitude and season.

In estimating the resulting damaging UV dose rate (DUV) for erythemal and DNA action spectra, we consider both a globally uniform ozone depletion consistent with the predictions of one-dimensional models, and latitude/season-dependent depletions as predicted by the two-dimensional model of Pyle<sup>5</sup>. Extensive calculations of this type have already been performed during the US Climatic Impact Assessment Program (CIAP) (see ref. 7). Our new calculations use a highly efficient and versatile radiative transfer code8 in which we incorporated a detailed data base for realistic atmospheres and which allows the computation of solar irradiance spectra to a predetermined degree of accuracy. Up to 70 km altitude we consider molecular and aerosol extinction and scattering profiles and include all

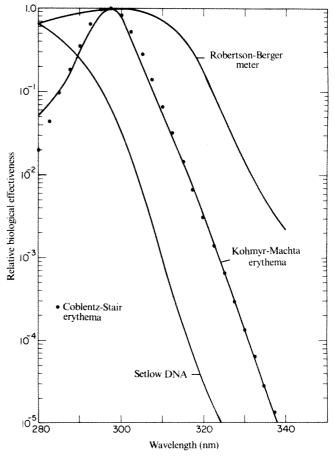


Fig. 1 Action spectra in use for biological effects of UV radiation.

orders of scattering in our computations. Our atmosphere model allows us to extend the results of Green and Mo<sup>7</sup> to larger latitudes.

We calculate first the downward solar flux at ground level for the biologically effective solar UV spectral region from 0.28 to 0.34  $\mu m$  (UV-B) for ozone depletions of 1-20%. Molecular absorption and scattering coefficients were prepared for five one-dimensional atmosphere models which correlate latitude and season9: (1) tropical; (2) midlatitude summer; (3) midlatitude winter; (4) subarctic summer; and (5) subarctic winter. Although the use of only five model atmospheres is obviously less detailed than a full two-dimensional model, this data base has proved to be adequate in extensive transmittance and radiance calculations as verified against laboratory and satellite measurements  $^{10}$ . The wavelength dependency of the ozone absorption coefficient is described by an analytical representation also used by Shettle and Green  $^{11}$ . We include absorption and scattering by aerosols which are assumed to be distributed according to a rural model profile  $^{12}$ .

The biologically damaging effect due to UV-B radiation reaching the ground may be quantified by the DUV dose rate calculated from

$$DUV = \int_{0.28 \mu m}^{0.34 \mu m} E(\lambda) I(\lambda, Z_0) d\lambda$$

where  $I(\lambda, Z_0)$  is the irradiance at ground level for the solar zenith angle  $Z_0$ , as computed from a radiative transfer calculation.  $E(\lambda)$  is the action spectrum characterizing a specific biological effect. Although changes in atmospheric ozone may produce rather small changes in the total solar flux reaching the ground, the changes in the UV-B part of the spectrum can be of considerable biological significance. The relative effectiveness of different wavelengths (the action spectrum  $E(\lambda)$ ) for most biological UV effects increases with decreasing wavelength. This function  $E(\lambda)$  must therefore be used as a weighting function for the solar irradiance when biological effects are to be estimated, as shown above for DUV. As can be seen from Fig. 1, the wavelength dependence of  $E(\lambda)$  is significantly different for biological effects<sup>2</sup>. Chemical compounds in biological systems that absorb UV-B radiation are mainly proteins and nucleic acids. For our calculations we chose as action spectrum for human sunburn the standard erythemal action spectrum<sup>13</sup> of Coblentz and Stair. The spectral sensitivity curve of the Kohmyr and Machta<sup>14</sup> modified Dobson instrument, used by Pyle and Derwent<sup>6</sup> as erythemal weighting function, is very similar to the Coblentz-Stair action spectrum, as shown in Fig. 1. As the action spectrum for human skin cancer is not known, we use the generalized DNA action spectrum compiled by Setlow11 generic representation for this damaging UV-B effect. The stronger response to shorter wavelengths of Setlow's DNA action spectrum as compared with the erythemal action spectrum leads to different results for the calculated DUV rates and RAFs. For completeness we also reproduce in Fig. 1 the spectral response curve of the Robertson-Berger meter which is used in the existing network of worldwide UV dose measurements. However, as seen from Fig. 1, and as emphasized in ref. 2, the response characteristics of this meter are quite different than the erythemal and the DNA action spectrum.

As the solar irradiance at ground level depends on the solar elevation, an effective solar zenith angle  $Z_0 = \cos^{-1} \langle \mu_0 \rangle$  is determined as a daily average over the continuously varying zenith angles at given date and latitude:

$$\langle \mu_0 \rangle = \frac{1}{T} \int_{t_{\text{sunrise}}}^{t_{\text{sunset}}} \mu_0(t) \ dt$$

where T is the duration of daylight. Figure 2 shows a plot of  $Z_0$  against latitude and season. This allows us to correlate the calculated DUV dose for a given effective zenith angle  $Z_0$ , and a given atmosphere model, with latitude and season. The consistency of using the effective solar zenith angle  $Z_0$  has been checked against the procedure in which the DUV dose is taken at several zenith angles and then averaged over the day. The

daily DUV doses as obtained by these two different averaging methods differ by <5%, which we consider adequate for this investigation.

The RAF is defined as the ratio of the percentage increase in DUV dose to the corresponding percentage decrease in total ozone. Figure 3 shows our calculated RAFs on the two-dimensional grid of latitude and season.

Considering the estimated ozone depletion in the range 1-5% through 1992 due to continued use of CFCs, as calculated by Pyle and Derwent with a two-dimensional model (Fig. 1 in ref. 6), we obtain the contour plots for the RAF of Fig. 3 when the erythemal and DNA action spectrum is used. If we consider a global ozone depletion of 10% as an estimate of a steady state condition, we obtain RAFs between 2.1 and 2.5 for erythema and between 2.8 and 3.0 for DNA. In both cases the RAFs are larger than for the lower 1992 depletion estimates which indicates the non-linearity of the radiation amplification. Note from Fig. 2 that the ratio of increase in erythemally weighted UV-B dose to the decrease in total ozone amount is always greater than unity and quite close to 2 even in the equatorial region; this is in contrast to the results reported in ref. 6. Calculations by F. M. Luther (personal communication) performed after the present analyses and extensive verifications of our computational methods and data16 indicate that the differences between our results for RAF and those of Pyle and Derwent<sup>6</sup> may be due to the different atmospheric data bases and different erythemal action spectra used in the radiative transfer calculations. The uncertainty in our results introduced by the computational method is estimated to be at most 10% in RAF for all latitudes and seasons. In addition, the radiation amplification factor for DNA-weighted DUV is close to 3 and thus significantly larger than for erythemally-weighted DUV for all latitudes and seasons. As regards the damaging effects to the human population of increased UV radiation due to continued release of CFCs, the above results may be interpreted as follows. If a 5% global depletion of ozone is reached due to the continued release of CFCs, then an amplification of biologically

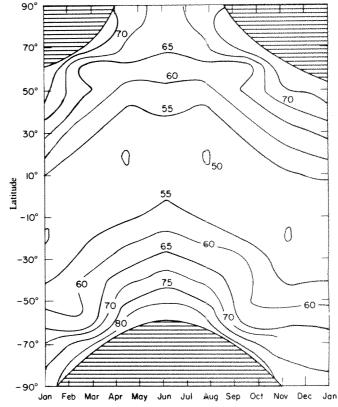
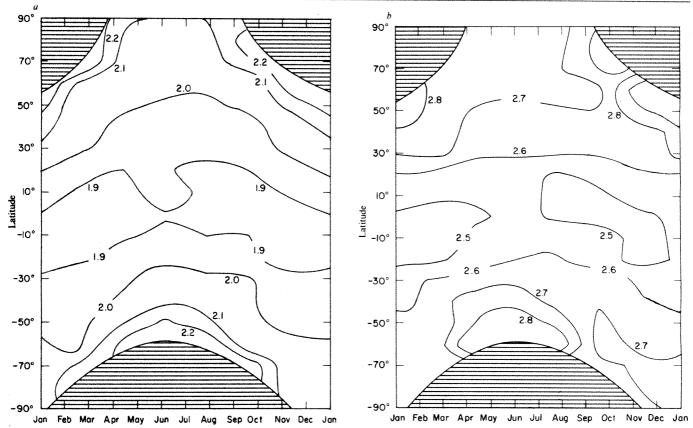


Fig. 2 Effective solar zenith angle determined as a daily average over continuously varying zenith angles at a given date and latitude. Shaded areas indicate polar regions for which no  $\langle \mu_0 \rangle$  is determined.



Radiation amplification factor for a, erythemally weighted and b, DNA weighted UV-B dose resulting from continued CFC usage through 1992. Percentage depletion in the total ozone taken after ref. 6.

damaging radiation should be expected that increases erythema ~10% (RAF ~ 2.0) and DNA damage by about 13%  $(RAF \approx 2.6)$ . The estimated increase of skin cancer rates is likely to be of the same order.

In conclusion, as our modelling indicates, the approximate value of 2 for the RAF should be replaced by a non-linear functional relationship; the larger the ozone depletion the larger the RAF. Plotted as a function of latitude and season, however, the RAF shows a fairly small gradient in all directions, consistent with the results of Green and Mo7. Using the latitude- and season-dependent ozone depletions estimated by Pyle and Derwent for continued CFC usage to the year 1992, we arrive at an RAF range between 2.5 and 2.8 for DNA-weighted UV-B dose and between 1.9 and 2.2 for erythemally-weighted dose. (These new results have been confirmed by I. Isaksen et al., unpublished data.) Note especially that our minimum RAF values are 1.9 (erythema) and 2.5 (DNA), which occur in the equatorial region.

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#### High species diversity and abundance of the epibenthic community in an oxygen-deficient basin

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Oxygen-deficient basins are common features of the ocean1. Studies of the response of benthic communities to low-oxygen conditions have previously been restricted to sediment infauna, and have reported mass mortalities during sudden oxygen depletion<sup>2-4</sup> and low species diversity during sustained oxygen deficiencies<sup>5,6</sup>. The deep epibenthic fauna of a 220-m deep fjord in British Columbia experiences annual conditions of low oxygen (<1.0 ml l<sup>-1</sup>). I have observed the reactions of the attached fauna to the changing oxygen levels during numerous dives in the submersible Pisces IV over 10 months, and report here that, despite records of the decimation of benthic invertebrate populations<sup>2-5,7-9</sup>, this deep-water assemblage maintains a higher diversity and animal abundance than does much of the upper photic zone. The tolerance developed by this community for a wide range of oxygen conditions, including periods of anoxia, has interesting implications for physiology, behaviour and community evolution.

The glacier-carved fjords of British Columbia are narrow and deep and frequently have restricted water circulation; the vertical sides provide sediment-free surfaces for the settlement of epibenthic organisms. Saanich Inlet, on Vancouver Island, is such a fjord, and has a maximum depth of 220 m. For much of the year the bottom waters are anoxic and anaerobic respiration is associated with the build-up of hydrogen sulphide<sup>1,11</sup>. The oxygen deficiency is moderated by an annual flushing which brings dense, oxygenated water over the shallow sill at the mouth from the nearby Strait of Georgia<sup>12,13</sup>.

Using the manned submersible Pisces IV (Institute of Ocean Sciences, BC), it was possible to study in situ the effects of an unstable anoxic layer on a hard substratum community in this fjord. Thirty-three dives were made from May 1980 to March 1981 throughout the Saanich Inlet. At the major study site (Fig. 1) the submersible ascended close to the cliff from 215 to 30 m. Water samples were collected in situ within 2 m of the cliff by a sampling system on the submersible and later analysed for dissolved oxygen. By extending the sampling tube, oxygen values at 0.05 m and 2 m off the rock face could be compared; no significant differences were found.

Photographs of the cliff were taken at 5-m intervals in depth using an external strobe light and an internal camera placed 1 m from a 30×30-cm quadrat extended from the submersible to touch the rock. The distributions and abundances of animals larger than 1 cm were determined for each photograph and the diversity calculated from the combined results. Species identifications were made from close-up photographs and verified from collections of loose rocks.

The dissolved oxygen structure for the sampling period is plotted in Fig. 2. Until late August 1980, the bottom 55 m of Saanich Inlet were anoxic. Dense, oxygenated water downwelled into the bottom layers and pushed a wedge of anoxic water upwards throughout September. In late October, the epibenthic fauna as shallow as 85 m was exposed to water with <0.1 ml  $O_2$  per l. Re-oxygenation ceased after November and by May 1981 the bottom waters developed patches of anoxia.

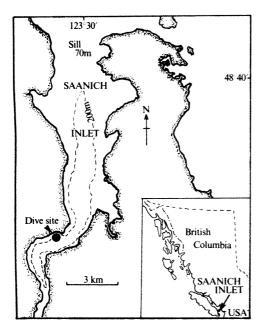


Fig. 1 Location of submersible dive site in Saanich Inlet, British Columbia. Deep-water circulation is restricted by the sill at the mouth of the inlet. In the south the walls are nearly vertical, exposing a silt-free gneissic substratum. Typical summer salinities range from 28.5% at the surface to 31.2% at 200 m; freshwater run-off into the inlet is minimal, the greatest influence being from the Cowichan River north of the sill from which intrusions into the inlet can be measured 13. Suspended sediment load is correspondingly low, although dense turbid layers due to plankton may form 13. Tidally influenced currents result in shallow-water exchange over the sill 17 and these daily fluctuations are detectable in the middle of the inlet but at velocities that rarely exceed 10 cm s<sup>-1</sup> (data not shown). The euphotic zone, defined by light levels >1% of that at the surface, was <20 m in the spring.

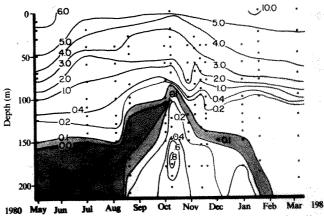
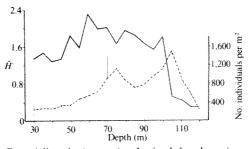


Fig. 2 Dissolved oxygen contours in ml O<sub>2</sub> per 1 for Squally Reach, Saanich Inlet, British Columbia. Sample points represent water drawn both by surface casts and by Pisces IV submersible; in the latter case, samples were fixed immediately inside the submersible and later analysed by the modified Winkler technique. Shading indicates anoxic zone. The disruption of contours from September to November 1980 is due to deep-water renewal by dense, oxygenated water entering the inlet over the sill.

Photographs and examination of samples in May 1980 showed the rock from the bottom to 130 m to be bare of macroscopic life. Small white sponges (Suberites simplex) appeared at 130 m and galatheid crabs (Munida quadrispina) at 127 m. In October, the anoxic layer was located between 100 and 130 m (Fig. 2). Tube worms were contracted, brachiopods closed and anemones limp. Dense populations of crab were found at 85 m (B. Burd, personal communication) where they had retreated as the anoxic water rose. By November brachiopod shells and crab carapaces were seen on ledges below 100 m; a few encrusting sponges were disintegrating and fragments of ascidean tunics were present between 85 and 100 m. Despite these observations, comparison of photographs taken above 120 m before and after the anoxic rise detected no significant changes in abundance of non-motile fauna. However, no epifauna were observable from the submersible between 130 and 120 m.

Two peaks in animal abundance (Fig. 3) were observed at 100 m and 75 m. At the lower depth, 84% of the individuals were sponges; at 75 m, 75% were ascideans. Numbers steadily declined towards the top station at 30 m and cover was correspondingly low. Above 30 m a distinct algae-dominated shallow-water fauna was present.

Diversity (Fig. 3) between 85 and 100 m was surprisingly great in view of the prevailing low-oxygen conditions. At 60 m it



**Fig. 3** Faunal diversity (----) and animal abundance (----) as measured from photographs from the submersible.

$$\hat{H}' = \sum_{i=1}^{n} p_i \ln p_i$$

(Shannon-Weaver diversity index)<sup>18</sup>. Four photographs were used for each depth from submersible ascents on the same location in September and November 1980, and February and late March 1981; qualitative comparisons were made with photographs taken without the quadrat in May 1980. Pictures were taken only where the rock was within 15° of vertical and the submersible could manoeuvre close to the rock. The resulting photographs of the 30×30-cm quadrat were readily reproduced to life size and so analysed for animals larger than 1 cm.



Fig. 4 Photograph from Pisces IV at -85 m. Here, the epifauna is dominated by ascideans but brachiopods, sponges and hydroids can also be seen.

marked the point at which animals of both shallow-water and high-oxygen dependence overlapped with the deeper, more tolerant species. In the photic zone diversity dropped with the appearance of encrusting red algae at 50 m. More than 40 species of epibenthic animals were identifiable from the submersible between 120 and 85 m and many more from rock samples. Phyletic diversity was high in this zone; 10 phyla were represented, including 16 classes. Conversely, infaunal assemblages of oxygen-poor areas were mostly impoverished5-

The oxygen levels at which the greatest faunistic changes are reported to occur are 1.5 and 1.0 ml l-1 (refs 6, 9). Despite < 1.0 ml l<sup>-1</sup> of oxygen for half the monitored time, the zone between 85 and 100 m had neither low diversity nor small populations (Fig. 4); even shelled animals such as brachiopods were common, despite possible physiological dissolution of carbonate6

The flushing of Saanich Inlet and the rise of anoxic water is an annual event  $^{1.13,14}$ ; 0.5 ml  $O_2$  per l has been recorded as shallow as 60 m. Elsewhere, similar influxes of low-oxygen water have resulted in the demise of large areas of benthic fauna<sup>3,4,7</sup>. Either a benthic community repeatedly re-establishes itself after oxygen deficiency<sup>5,7</sup> or it becomes highly tolerant of changing oxygen conditions. The latter seems to be the case in Saanich Inlet; the community maintained a stable constituency, noticeable mortality occurred only in areas of sustained anoxia and throughout the 10 months, there was no significant recruitment to any taxon.

The persistence of this community is pertinent to (1) the behavioural and physiological adaptations that allow survival in very low-oxygen conditions; (2) the possible regulation of competitive dominance 10,15 in the substrate-limited depths by periods of anoxia that cause some mortality; and (3) the evolution of community structure during the Palaeozoic when atmospheric levels of oxygen were low 16—the composition and behaviour of the deeper part of this community may reflect patterns in early Cambrian assemblages.

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#### Epithelial cells of Hydra are dye-coupled

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In the past decade, a strong correlation has been established between gap junctions, seen in cell ultrastructure studies, and cell coupling (ionic, metabolic or dye coupling) assayed physiologically1. In Hydra, ultrastructural analyses have indicated that the epithelial cells of both cell layers are connected extensively by gap junctions; gap junctions have also been observed between the two layers<sup>2-7</sup>. On the basis of these results, one would expect electrical and dye coupling between epithelial cells of Hydra. However, de Laat et al.8 reported that these cells were neither dye- nor electrically coupled, which was unexpected as cells in another coelenterate have been shown to be coupled ionically9. Cell-cell coupling in Hydra is particularly interesting because extensive experiments on head regeneration in this coelenterate have led to well-defined models of patterning that require communication between cells of the type that may be provided by gap junctions 10-14. We have re-examined dye coupling in Hydra and we report here that, after injection of Lucifer yellow into single epithelial cells, neighbouring cells were observed to contain the dye. We conclude that the epithelial of cells of Hydra are indeed dye-coupled.

The body column of Hydra consists of two layers of epithelial cells, the epidermis and the gastrodermis, arranged in a cylindrical shell with the epidermis on the outside and the gastrodermis lining the gastric cavity. The cells of both layers possess large vacuoles; results of light and electron microscopy studies suggest that these vacuoles occupy less than half the cell volume <sup>15,16</sup>. By fixing the animals in isotonic solutions instead of the typical hypertonic solutions, it has been shown (R. D. Campbell, personal communication) that the vacuole actually comprises a much greater fraction (perhaps 80-90%) of the cell volume. The cytoplasm is therefore often only a thin shell 1-3 µm thick between the plasma membrane and the vacuoleelectrophysiological properties support this arrangement. On penetrating an ectodermal epithelial cell with a glass microelectrode, several investigators have found a transient negative membrane potential, followed by a stable positive potential 17-1 Chain 19 reported that the electrode could then be moved large distances into the cell without altering the positive potential, consistent with the electrode being within the vacuole. Movement of the animal has also hindered intracellular electrophysiological investigations of *Hydra*. During impalement, movement of only a few cell diameters is sufficient to interfere with measurements. Impaling an otherwise quiet *Hydra* can induce movement, and use of nerve-free *Hydra*<sup>20,21</sup>, which are not moving on a macroscopic scale, is of little help as they still make small movements. Thus the delicate process of penetrating and remaining in the thin shell of cytoplasm is made even more difficult.

To increase the likelihood of positioning the electrode in the cytoplasm of the cell, we used the following techniques. Movement of *Hydra* was eliminated by: (1) Removing the head and foot of the animal, which greatly reduced stretching and contraction of the body column. (2) The body column was threaded onto monofilament fishing line having a diameter slightly larger than that of the body column. The column easily stretched circumferentially to accomodate the larger diameter. (This is a standard technique used in *Hydra* grafting experiments <sup>13,14,22,23</sup>.) (3) Animals were starved for 2–4 days (*Hydra* can starve for 10–15 days and still remain healthy). To reduce vacuole size, animals were acclimatized to 50 mM sucrose in *Hydra* medium for 18–24 h, which does not affect the health of the animal <sup>18</sup>. With these treatments, stable negative or positive membrane potentials were readily obtained.

Epithelial cells were impaled with micropipettes containing Lucifer yellow and filled with the dye iontophoretically. While developing methods to optimize cell impalement, we observed that the cells are coupled in a wide variety of conditions (normal, sucrose and hypertonic medium), which indicates that coupling of the cells is not an abnormal state induced by our manipulation of the conditions. Of the 121 cells that were impaled, 44 were located by fluorescence microscopy; for 19 of these, Lucifer yellow was found in at least one neighbouring cell. Presumably, a large number of cells could not be found due to cell death.

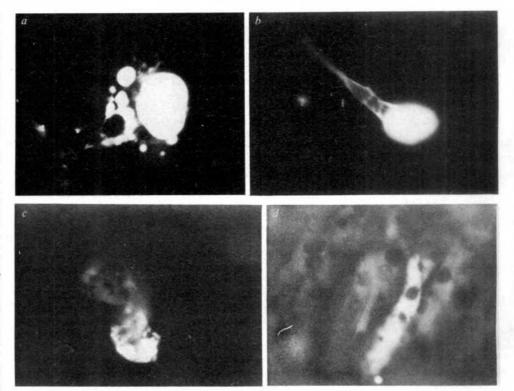
Occasionally, cells lysed while being observed in the fluorescence microscope. No residual dye remained after such lysis and the neighbouring cells did not pick up any dye.

In subsequent experiments using the improved methods described above, 86 cells were impaled and filled with Lucifer yellow: 45 of these were located by fluorescence microscopy, and 25/45 cases demonstrated passage of the dye to one or more neighbouring cells. Four examples of dye-injected cells are shown in Fig. 1. In all cases, the filled cells were epithelial cells, as opposed to the much smaller cells found in the interstices between epithelial cells. They were of the same size and shape as epithelial cells and often had long processes extending away from the cell, which were probably muscle processes found at the base of each epithelial cell type<sup>24</sup> (see Fig. 1b).

Two distinct patterns of dye distribution were observed. In one, the cytoplasm was uniformly, intensely stained, while several round dark spots (assumed to be vacuoles and the nucleus) were not (Fig. 1d). In the other pattern, one of the vacuoles was very intensely stained while the remainder were not. In these cells, the cytoplasm was much less intensely stained (Fig. 1a, b). Although no dye passage was seen in these cases (Fig. 1a, b), dye coupling was observed for both types of filled cell (Fig. 1c, d). The vacuoles of the cells coupled to the injected cell were always dark compared with the cytoplasm. Coupling was observed both within each cell layer and between cell layers.

Two types of membrane potential were also found. In some cases, the membrane potential was negative  $-10\pm 6\,\mathrm{mV}$  ( $\pm$  s.d.) before, during and after filling the cell with Lucifer yellow. In other cases, a positive membrane potential was obtained ( $+21\pm 8\,\mathrm{mV}$ ) before, during and after dye filling. Occasionally, the initial potential was negative, and suddenly switched to positive during dye filling. The reverse switch was never observed. These potentials are probably not an exact measure of the

Fig. 1 Sample micrographs of Hydra cells filled with Lucifer vellow. a, b, A region with positive membrane potential was filled with the dye and no passage occurred. In a, there was no leakage of dye from the vacuoles; in b, the cytoplasm was clearly labelled as well as the single bright vacuole. Presence of Lucifer yellow in the cytoplasm was always easily recognized by the fine cell processes that were filled with the dye. The vacuole in a fragmented into many smaller vacuoles during observation, before the photograph was taken. c, Dye passage in a region of positive membrane potential which was dye filled. A bright apical vacuole was observed in the cell, with considerable amounts of dye in the surrounding cytoplasm. The dye passed to two cells, one of which is in the plane of focus. d, A region of negative membrane potential that was dye filled. The vacuoles in all these cells were dark. The dye passed to many other cells, extending beyond the limits of the photograph. The cell shape observed in a-d varies because the shape of the animals at the time of observation. Cells were identified (epidermis as opposed to gastrodermis) by their plane of focus with respect to the mounted stenoteles, which are know to be on the outer surface of Hydra 30. Hydra attenuata were maintained in Hydra medium (1 mM CaCl<sub>2</sub>, 1 mM NaHCO<sub>3</sub>, 10 µM EDTA), and were starved for 2-4 days before an experiment. For the 18-24 h preceding an



experiment, the medium was supplemented with 50 mM sucrose (to reduce vacuole size). The head and basal disk of the animal were removed and the resulting body column threaded on to a monofilament fishing line (0.25 mm diameter). The fishing line was secured with dental wax in the centre of a Petri dish filled with sucrose–Hydra medium (with body column parallel to the dish bottom). The dish was mounted on the stage of a Hoffman modulation microscope (viewed at ×280); illumination was supplied by a fibre optic light source placed outside the Faraday cage, with heat filters and a red filter positioned in the light path to avoid damaging the Lucifer yellow-filled cells. Glass micropipettes (200–800 Ω) were pulled from Ultratip glass (Haer) and black-filled with 3% Lucifer yellow CH (Aldrich). Cells were impaled using a piezoelectric bending element which withdrew the electrode slightly (2 μm) and then allowed it to snap forward, thus penetrating the cell membrane. Lucifer yellow was introduced into the cell iontophoretically with 0.5 nA hyperpolarizing current pulses (1 Hz, 50% duty cycle) for 15 s. The animals were removed from the fishing line and allowed to recover in the dark for 10–60 min, after which time they were pressed between two microscope coverglasses in a small amount of sucrose–Hydra medium. A small bead of silicone grease (Dow-Corning) placed between the two coverglasses prevented the animals from being overly flattened. This 'sandwich' arrangement could be flipped to view both sides of the animal. The animals were observed and photographed (×325) with a Zeiss UEM epifluorescence microscope through a Zeiss filter set designed for Lucifer fluorescence (487704).

Table 1 Correlation between membrane potentials and staining patterns of Hydra epithelial cells

***************************************			
Staining pattern	Type of membrane $-/ (n = 38)$	,	(initial/final) +/+ (n = 38)
Cells that could be located	7	9	29
a, Bright cytoplasm and dark vacuoles	7	2	5
No. of cells coupled	4	1	3
b, Bright vacuole and dim cytoplasm	0	7	21
No. of cells coupled	-	4	10
c, Ambiguous No. of cells coupled	0	0	3 2

Data from the final set of animals in which the membrane potential was measured before, during and after the injection of Lucifer vellow. The cells were grouped together by the membrane potentials before and after injection. Techniques used are described in Fig. 1 legend. The cytoplasm of all five samples with positive, positive potentials (+/+)and one with negative, positive potentials (-/+) in a were much less intensely stained. This low level of observed dye could be due to dye passage from a filled cell which subsequently lysed, accounting for the cytoplasmic dye location. In three of these six cases, fluorescent debris was observed around the dimly fluorescent cells.

membrane potential because the size of the electrode tip potential is unknown. A contribution from the tip potential could be substantial due to the large difference between the ionic strength of Hydra medium (5 mM) and that of the cytoplasm (120-150 mM) (ref. 25 and H.R.B., unpublished results). Nevertheless, two distinct cell regions of differing membrane potential were consistently observed throughout our experiments.

A correlation was observed between the staining patterns and membrane potentials (see Table 1): intensely stained vacuoles and dimly labelled cytoplasm were correlated with positive potentials. These results are consistent with the positive potential resulting from penetrating a vacuole. The appearance of dim fluorescence in the cytoplasm of the positive potential fills indicated that some leakage of the dye occurred during and/or after dye filling, perhaps when the electrode was withdrawn. Although there were equal numbers of cells filled with dye for each type of membrane potential, a much smaller fraction (18% as opposed to 76%) of the 'negative, negative' fills were later located, suggesting a higher probability of cell damage when impaling the cytoplasm.

It seems likely that in previous work8, which recorded positive membrane potentials for many of the cells impaled, the electrode was placed in the vacuole, as we found a strong correlation between positive potentials and dye filling of the vacuole. This may have greatly reduced the chance of observing ionic or dye coupling. In addition, cell damage or cell death may have uncoupled the cells. Approximately half the cells that were successfully filled with dye in our work showed no dye passage, which we take to indicate the ease with which the cells may be damaged.

Our results clearly indicate that the epithelial cells of Hydra are dye-coupled, probably through gap junctions, as has been well established in the epithelia of other animals. This finding is of particular interest in Hydra in terms of pattern formation. Two developmental gradients, one of activation and one of inhibition, are involved in the formation of the head at the apical end<sup>8,10,14,26</sup>, and a further two gradients control foot formation<sup>10,13,14</sup>. Indirect evidence suggests that the inhibition gradients are due to diffusible substances<sup>13,27,28</sup>. Recently, it has been shown that the epithelial cells are responsible for transmission of the head inhibition down the body column<sup>29</sup>. A logical channel for the movement of such a diffusible substance would be through the gap junctions of the epithelial cells.

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#### Protein phosphatase-1 is involved in Xenopus oocyte maturation

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Two key steps in meiotic maturation of the Xenopus oocyte involve protein phosphorylation-dephosphorylation<sup>1</sup>. A burst of cyclic AMP-independent phosphorylation occurs at the time of the breakdown of the nuclear envelope, whether maturation is triggered by progesterone, by the inhibitor of cyclic AMP-dependent protein kinase (PKI)<sup>2</sup> or by the maturation promoting factor (MPF)<sup>1,3</sup>. Also, an *in ovo* decrease in the level of the free catalytic (C) subunit of cyclic AMP-dependent protein kinase, induced by microinjection of pure PKI<sup>4</sup>, initiates meiotic maturation<sup>1,2,4</sup>. This suggests that MPF appears only after dephosphorylation of a phosphorylated maturation protein Mp-P<sup>4</sup>. We now show, by microinjection of pure inhibitor-1 of protein phosphatase-1<sup>5</sup>, which blocks progesterone- and PKI-induced maturation but not the MPFinduced one, that protein phosphatase-1 catalyses the dephosphorylation of Mp-P.

Phosphoinhibitor-1 was injected into Xenopus oocytes to a final concentration of 15 µM. Microinjected oocytes maintained in saline remained morphologically healthy and resumption of meiosis never occurred. When exposed to  $1 \mu M$  progesterone 4 h after microinjection of inhibitor-1, they matured with the same efficiency and kinetics as controls, showing that inhibitor-1 is neither an inducer of maturation by itself nor toxic to the cell. Oocytes were also stimulated by 1 µM progesterone at various times before and after the microinjections (Fig. 1). Inhibitor-1 proved to be a potent inhibitor of maturation, having maximal efficacy when injected 1 h after incubation with the steroid. In contrast, microinjection of inhibitor-1 3 h after progesterone

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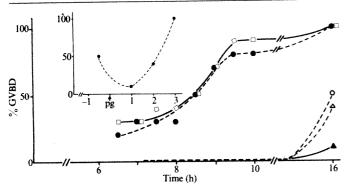


Fig. 1 Comparison of kinetics and % maturation after treatment with progesterone and phosphatase inhibitor-1. Ten oocytes were exposed either to 1 μM progesterone only (•) or to 1 μM progesterone 30 min after (○), 1 h before (△), 2 h before (△) or 3 h before ( ) inhibitor microinjection. Inset shows the time dependence of inhibitor efficacy: % of germinal vesicle breakdown (GVBD) 16 h after exposure to progesterone (pg) versus time of microinjection. Fully grown oocytes (stage VI11) were defolliculated as described elsewhere<sup>4</sup>. Inhibitor-1 of phosphoprotein phosphatase-1 was purified according to Nimmo and Cohen<sup>5</sup> and phosphorylated<sup>9</sup> by the pure catalytic subunit of cyclic AMP-dependent protein kinase 12. Phosphoinhibitor-1 was freed from the catalytic subunit and MgATP by heat treatment and dialysis with 60 mM ammonium bicarbonate, lyophilized and dissolved in a 5 mM MES (2(N-morpholino) ethane sulphonate) buffer pH 7.0, containing 1 mg of serum albumin per ml. Microinjection at the equator level of 50 nl of a 100  $\mu M$  solution yielded an intracellular concentration of 15  $\mu$ M, assuming a diffusion compartment of 0.3  $\mu$ l (ref. 4). As inhibitor-1 was calculated to be 1.5 µM in skeletal muscle<sup>5</sup>, microinjected inhibitor-1 should block all protein phosphatase-1 present. Its dephosphorylation into the inactive dephospho form is indeed prevented by the high free C subunit level of the resting oocyte4. The criterion for maturation was the appearance of a white spot at the animal pole of the oocyte. This experiment was done using oocytes from the same female; similar results were obtained with oocytes from two other females.

stimulation failed to alter the rate and extent of maturation, suggesting that inhibitor-1 blocks the steroid-induced maturation at an early step, without interfering with the effects of MPF. Indeed, microinjection of MPF into oocytes already injected with inhibitor-1 induced normal maturation (Fig. 2). The site of action of inhibitor-1 in the events triggered by progesterone was more precisely delineated by inducing maturation with pure PKI4. Inhibitor-1 inhibited the effect of subthreshold doses of PKI (≤0.3 µM) while maturation induced by higher PKI concentration was only delayed (Fig. 3).

These findings confirm the four-step scheme of meiotic maturation proposed previously4: on reassociation of regulatory and catalytic subunits of cyclic AMP-dependent protein kinases, resulting from the progesterone-induced fall in the cyclic AMP levels, a phosphorylated maturation protein Mp-P is dephosphorylated to the active form Mp, which triggers the synthesis of

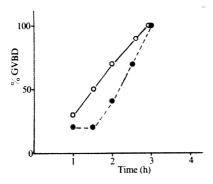


Fig. 2 Comparison of kinetics and % maturation after microinjections of phosphatase inhibitor-1 and MPF. Ten oocytes were injected either with MPF ( ) or with inhibitor-1 and then immediately with MPF (O).

MPF. If this scheme were correct, progesterone- and PKIinduced maturation, but not the MPF-induced one, should be blocked by phosphatase inhibitors, as shown here to be the case. The efficacy of inhibitor-1 shows that the putative Mp-P is a substrate of its specific target, protein phosphatase-1, and that a type-2 phosphatase cannot be the major enzyme involved in Mp-P dephosphorylation<sup>6</sup>. This confirms previous reports of protein phosphatase-1 in a number of tissues and its catalysis of the dephosphorylation of various substrates belonging to different metabolic pathways7. It also eliminates initiation factor eIF-2 from this step in maturation because eIF-2 phosphatase was recently shown to be a type-2 phosphatase8. However, the remote possibility that inhibitor-1 blocks maturation by an unknown indirect mechanism cannot be excluded, even though protein phosphatase-1 is the only known target of inhibitor-1.

The fact that inhibitor-1 is more efficient when injected 1 h, rather than 2 h after the exposure to the steroid, indicates a slow, rate-limiting dephosphorylation of Mp-P into Mp, resulting from the balance between reduced protein kinase activity and a phosphatase activity that is either constant or enhanced by the dephosphorylation of endogenous inhibitor-1.

That oocytes escape at least in part the inhibitor-1-induced maturation blockade after a prolonged time is readily understood from the fact that inhibitor-1 does not inhibit its own

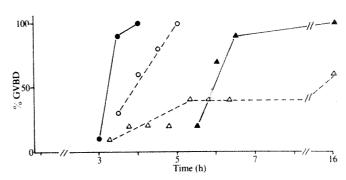


Fig. 3 Comparison of kinetics and % maturation after phosphatase inhibitor-1 and PKI treatment. PKI was microinjected 1 h after microinjection of inhibitor-1. Ten oocytes were injected either with 15 µM inhibitor-1 and 1.5 µM PKI (○) or with 15 µM inhibitor-1 and 0.3 µM PKI (△) or with 1.5 µM PKI (●) or with 0.3 µM PKI (▲). This experiment was done using oocytes from the same female; similar results were obtained with oocytes from two other females. PKI was prepared and injected as described previously<sup>4,13</sup>.

dephosphorylation by protein phosphatase-19. Microinjection of high concentrations of PKI only delays maturation because the total inhibition of the catalytic subunit of cyclic AMPdependent protein kinase leads to rapid inactivation of inhibitor-1 by protein phosphatase-1 which dephosphorylates the inhibitor into its inactive dephospho form10

This work also raises the intriguing possibility that inhibitor-1 is the maturation protein per se, although there is no experimental evidence for such identification.

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## Perivascular infiltrates of leukocytes in brains of scrapie-infected mice

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Four chronic subacute spongiform encephalopathies—Kuru and Creutzfeld-Jakob diseases of man, transmissible encephalopathy in mink and scrapie in sheep—are caused by slow viruses with unconventional biological and biochemical properties<sup>1-4</sup>. Since the transmission of the scrapie agent to mice<sup>5</sup> and the comparative histological analysis of Kuru in man<sup>6</sup> and of scrapie in the mouse, scrapie-mouse systems have become important models of spongiform encephalopathies. The predominant histopathological lesions (for review see refs 2, 4) found in these diseases result from nerve cell degeneration<sup>7</sup>. It seems that, in contrast to diseases of the nervous system caused by conventional viruses, perivascular leukocyte infiltrations do not play any part in the progression of spongiform encephalopathies and it is not known whether leukocyte infiltrations can be induced by unconventional slow viruses. Here we report that scrapie infection can indeed produce such lesions in mice.

Perivascular cuffs have occasionally been found in brains of patients with Kuru<sup>1,2</sup>, but there are conflicting data on scrapie. Perivascular cuffs have been reported to be absent from histological sections of brains from scrapie-diseased animals, but present in intracerebrally (i.c.) injected scrapie- and mockinfected goats, and in brains of sheep injected i.c. with scrapie agent<sup>10</sup>. However, no controls were used in the latter study. Zlotnik and Rennie<sup>11</sup> observed similar lesions in naturally infected sheep but also in control animals which were apparently healthy. Therefore, they did not consider these lesions to be associated with the disease.

We first studied scrapie in inbred STU mice<sup>12</sup> using mouse brain containing Chandler agent (47th passage; from Dr R. Kimberlin). When injected i.c.<sup>13</sup> and passaged in our mice, this material gives incubation periods of ~150 (10<sup>-2</sup> dilution of brain) to 240 (10<sup>-7</sup> dilution) days. Examination of haematoxylin-eosin-stained sections of brains from mice infected i.c. with scrapie material revealed perivascular cuffs in some sections of a

few animals at the clinical stage of the disease (Fig. 1a). We found these lesions only when the whole brain was sliced and five 10- $\mu$ m sections taken every 200  $\mu$ m. We therefore used this technique to analyse brains of age-matched, mock-infected and scrapie-infected animals for perivascular cuffs. Aliquots (50  $\mu$ l) of a  $10^{-2}$ - $10^{-3}$  dilution of brains from scrapie-diseased or healthy mice were injected intraperitoneally (i.p.), to avoid any possible immune reaction to brain injury. The mice developed symptoms of clinical scrapie 170-200 days after infection.

In the first experiment, only animals infected with scrapic material were used and histology showed that 5/10 developed perivascular cuffs (Table 1). Four animals were scored as positive in the advanced incubation period of the disease, at days 150–192. Surprisingly, however, one animal was scored as positive at day 73 after infection when less than one-half of the incubation period had passed and clinical and histological diagnosis of scrapic was not possible. However, titres of the scrapic agent have been reported at this stage for Compton white mice<sup>14</sup>.

In experiment 2, coded brains of control and infected mice were examined 'blind'—without the histologist knowing the number of infected brains; 4 of 14 brains of scrapie-infected animals were scored as positive whereas no control animals showed perivascular cuffs. Again, in one mouse brain infiltrates were detected in the early phase of the incubation period, at day 63.

Experiment 3 examined only advanced cases of scrapie, with the result that even in severely sick animals, only a limited number of mice (4/10) showed lesions. However, note that our technique of serial sectioning allowed histological examination of only one-fifth of the total brain. Control animals were free of infiltrations.

We have no evidence that the perivascular cuffs observed could be related to a possible contagious agent in the mouse stock as: (1) STU mice are no more susceptible to infections than conventional inbred mouse strains: (2) infected and mockinfected animals were kept in the same room; and (3) the experiments were performed at different times as indicated in Table 1. (4) No mice died during the experiment and none looked sick before clinical symptoms of scrapie appeared. (5) In experiments 2 and 3, the spleens were removed and the weights of single spleens recorded. There was no indication of a splenomegaly related to the perivascular cuffs.

Because of the long incubation period, even after i.c. injection, and as perivascular cuffs also developed in an animal

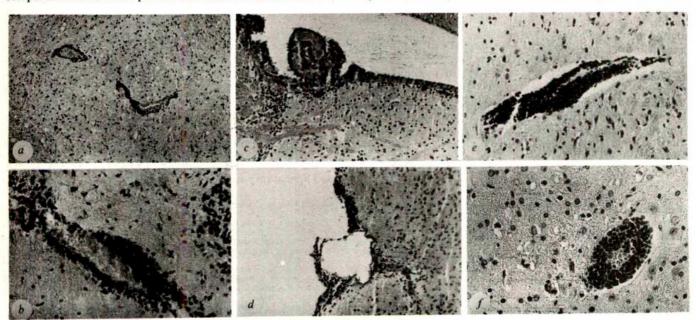


Fig. 1 Examples of meningeal leukocyte infiltrations and perivascular cuffs in scrapie-infected mouse brains. a, Perivascular cuffs 138 days after i.e. injection (×58); and b, 63 days after i.p. injection (×133). c, Meningeal infiltration 73 days after i.p. injection (×85); and d, 147 days after i.p. injection (×85). e, Perivascular cuffs 163 days after i.p. injection (×133); and f, 204 days after i.p. injection (×217).

**Table 1** Leukocyte infiltrations in mouse brain after i.p. injection of  $10^{-2}$ – $10^{-3}$  dilutions of brain material from healthy or scrapie-diseased mice

	No. of animals injected		Days post-injection on which animals were killed for	No. of animals with leukocyte infiltrations	
Expt	Control	Scrapie	histology	Control	Scrapie
1 (1979)		10	25, 56, 73(+), 120, 132, 150(+), 170(2)(+), 178(+) 192(+)	,	5/10
2 (1980)	21	14	20, 35, 42, 56, 63(+), 70, 77, 91, 105, 119, 133, 147(+), 161(+), 176(+)	0/21	4/14
3 (1980)	4	14	190, 204	0/4	4/14
4 (1981)	12*	12*	65(12), 71(+)(12)	0/12	2/12

Numbers in parentheses in column 4 indicate numbers of animals taken for histology. Animals in which perivascular cuffs were found are indicated by (+). All histology was done using coded material.

Animals received brain material treated with formalin

which received an i.c. inoculum of a 10<sup>-7</sup> dilution of scrapie brain material, it is unlikely that our passaged material could have been contaminated by another contagious agent. Nevertheless, we tried to exclude this possibility by exploiting the high degree of resistance of the scrapie agent to formaldehyde15 To destroy any other infectious agents, a low speed (500g for 5 min) supernatant of a 10<sup>-1</sup> brain homogenate was pretreated with 8% formalin at room temperature for 16 h and then injected  $(10^{-3} \text{ dilution})$  into mice. As in experiment 2, coded brains were scored for perivascular cuffs 65 and 71 days after infection, when diagnosis of scrapie was not possible. All controls were free of perivascular cuffs, whereas 2/12 animals of the group which had received scrapie-infected material were positive. Examples of leukocyte infiltrations at various times after i.p. injection are shown in Fig. 1; we have selected mild (Fig. 1c, d) and more severe (b, e, f) infiltrations to indicate the range of our scoring. Perivascular cuffs have been observed mainly in the hippocampus and thalamus at very low frequency during the routine examination of many thousands of brains of several mouse strains infected with various different strains of scrapie (H. Fraser, personal communication).

Our results thus show that leukocyte infiltrations can be induced by an unconventional slow virus and that brains of healthy animals are free from these lesions; that in some cases perivascular cuffs can be detected relatively early in the latent period, when diagnosis of the disease is not possible, but that they do not occur in all infected animals, even in the clinical phase when the disease can be diagnosed by symptoms and histology. Thus it is possible that the apparently healthy control sheep observed by Zlotnik and Rennie<sup>11</sup> to contain perivascular cuffs but showing no clinical symptoms were animals harbouring the scrapie agent. Although our technique could not detect perivascular cuffs in all scrapie-infected animals, we suggest that these lesions are not incidentally associated with the disease but may be a consequence of a very weak virus-induced autoimmune response, as has been reported for Kuru and Creutzfeld-Jacob diseases<sup>17</sup>. However, it is also possible that the perivascular cuffs are caused by an immune reaction to a product of the scrapie agent which has extremely low antigenicity. Furthermore, our results indicate that this may also be the case for diseases of the central nervous system in man, in which perivascular cuffs are seen occasionally, but which are not known to be caused by an infectious agent.

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#### Immunization against blood-stage rodent malaria using purified parasite antigens

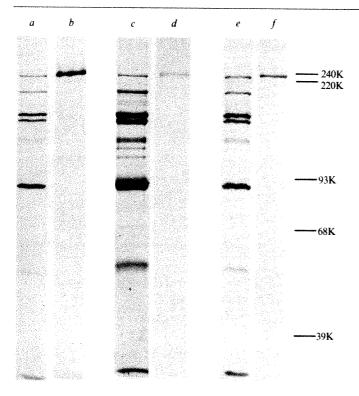
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We have reported the production of several hybridoma lines secreting monoclonal antibodies recognizing antigens of the blood forms of a rodent malaria parasite, Plasmodium yoelii yoelii<sup>1,2</sup>. Line WIC 25.77 secretes an IgG2a (antibody 25.77) which reacts specifically with merozoites in the indirect immunofluorescence (IIF) assay, and which was protective on passive transfer. Another of the hybridoma lines, WIC 25.1, secretes an IgG2a (antibody 25.1) which reacts with an antigen apparently associated with the membranes of schizonts, but this antibody was not protective on passive transfer. We have used our monoclonal antibodies to identify and purify antigens of P. y. yoelii, and we report here that mice immunized with either a 235,000 apparent molecular weight (MW) merozoite-specific protein or a 230,000 MW schizont protein and its proteolytic derivatives were protected against infection with P. y. yoelii.

Schizonts were obtained by centrifugation of P. y. yoeliiinfected mouse erythrocytes through a layer of Histopaque, and were metabolically labelled in vitro with 35S-methionine. The labelled schizonts (including a subpopulation of segmenters containing mature merozoites) were solubilized in a buffer containing a nonionic detergent. Immunoprecipitation of the extract followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the washed immune complexes and fluorography revealed that antibody 25.77 reacted with a single polypeptide of 235,000 MW whereas antibody 25.1 precipitated eight major polypeptides and several minor bands (Fig. 1a, b). The apparent MWs of the major polypeptides were estimated to be 230,000, 197,000, 160,000, 151,000, 126,000, 90,000, 56,000, and 28,000.

Preparative scale purification of these antigens was achieved by monoclonal antibody affinity chromatography. Washed y. yoelii-parasitized mouse erythrocytes were extracted in a buffer containing 1% (v/v) NP40 and protease inhibitors. After high speed centrifugation the supernatant was applied sequentially to two columns containing 25.77 antibody-Sepharose and 25.1 antibody-Sepharose, respectively. Specific elution of bound protein from the 25.77 antibody column was achieved at pH 8.0 with 2 M potassium thiocyanate and 50 mM diethylamine, pH 11.5, was used for specific elution of protein bound to the 25.1 antibody column. After a second cycle of absorption and elution the purity of the protein preparations was assessed by SDS-PAGE. Essentially, the only polypeptides present were



**Fig. 1** P. y. yoelii polypeptides analysed by SDS-PAGE<sup>7</sup> in 7.5% gels. In tracks a, b and e, f, the polypeptides are labelled with  $^{35}$ S-methionine and S-methionine and visualized by fluorography, and in tracks c, d, they are stained with Coomassie blue. Track a, products immunoprecipitated with antibody 25.1; track b, immunoprecipitation with antibody 25.77; track c, protein purified by monoclonal antibody affinity chromatography using antibody 25.1; track d, protein purified by monoclonal antibody affinity chromatography using antibody 25.77; track e, <sup>35</sup>S-labelled polypeptides immunoprecipitated by serum from mice immunized with the protein preparation shown in track c track f, products immunoprecipitated by serum from mice immunized with the protein preparation shown in track d. A schizont-enriched fraction of parasitized erythrocytes, prepared by centrifugation through a layer of Histopaque (Sigma)<sup>8</sup>, was labelled with <sup>35</sup>S-methionine as previously described<sup>2</sup>. Labelled (for immunoprecipitation) or unlabelled (for affinity chromatography) parasitized erythrocytes were extracted on ice in at least 5 chromatography) parasitized erythrocytes were extracted on ice in at least 5 volumes of a buffer containing 50 mM Tris-HCl pH 8.0, 5 mM EDTA, 5 mM EGTA, 5 mM iodoacetamide, 1 mM phenylmethyl sulphonyl fluoride, 0.1 mM TLCK, and 1% (v/v) NP40. Insoluble material was removed by centrifuging at 100,000g for 45 min at 4 °C. Formalin-fixed Cowan I strain Staphylococcus aureus organisms were used to precipitate immune complexes? For fluorographic visualization, gels were treated with En<sup>3</sup>Hance (NEN) before exposure at -70 °C using Kodak X-Omat H film. Monoclonal antibodies were purified from ascitic fluid from BALB/c mice carrying hybridoma lines WIC 25.1 and WIC 25.77 using Protein A-manufacturer's recommended method, and equilibrated with 10 mM Tris-HCI pH 8.0, 1 mM EDTA, 1 mM EGTA, 1% (v/v) NP40, at 4 °C. The 25.77 antibody-Sepharose column was washed with equilibration buffer containing 0.5 M NaCl then with 10 mM Tris-HCl pH 8.0, 1 mM EDTA,  $1~\rm mM~EGTA,\,0.01\%~NP40,$  and then bound protein was eluted using 2 M KSCN in the same buffer. The eluate was dialysed against 0.1%~NP40 and reapplied to the column. Retained material was re-eluted using 2 M KSCN,

0.01% NP40, concentrated and dialysed. The 25.1 antibody-Sepharose column was washed with equilibration buffer containing 0.5 M NaCl, then with buffer containing 0.1% NP40. Bound material was eluted with 50 mM diethylamine pH 11.5, 0.1% NP40. The eluate was dialysed against the pH 8.0 buffer containing 0.1% NP40, and applied a second time to the re-equilibrated column. The column was washed with 10 mM Tris-HCl pH 8.2, 1 mM EDTA, 1 mM EGTA, 0.5% (w/v) sodium deoxycholate, and the retained material was eluted with 50 mM diethylamine pH 11.5, 0.5% sodium deoxycholate, concentrated and dialysed. 400 µg of protein was routinely recovered from the 25.77 antibody-Sepharose column, and the 25.1 antibody-Sepharose column yielded some 1,600 µg of protein. Antisera against the purified P. y. yoelii proteins for immunoprecipitation analysis (tracks e, f) were raised in BALB/c mice as described in the Fig. 4 legend. Molecular weight markers were human spectrin heterodimer (240,000 and 220,000), phosphorylase b (93,000), bovine serum albumin (68,000) and aldolase (39,000).

identical to those previously detected in the monoclonal antibody immunoprecipitates (Fig. 1c, d).

The relationship between the polypeptides recognized by antibody 25.1 was revealed by pulse-chase labelling of *P. y. yoelii* schizonts with <sup>35</sup>S-methionine *in vitro*, followed by solubilization and immunoprecipitation of samples at various time intervals after labelling. The label was initially

incorporated into the 230,000 MW polypeptide, then during further incubation it sequentially appeared in the smaller polypeptides, with a concomitant reduction in the amount of label remaining in the 230,000 MW component (Fig. 2). Thus, the native antigen recognized by antibody 25.1 is a 230,000 MW protein and the smaller polypeptides are proteolytic fragments of it, each retaining the antigenic determinant recognized by the

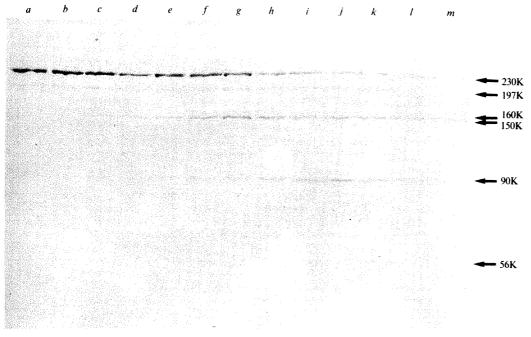
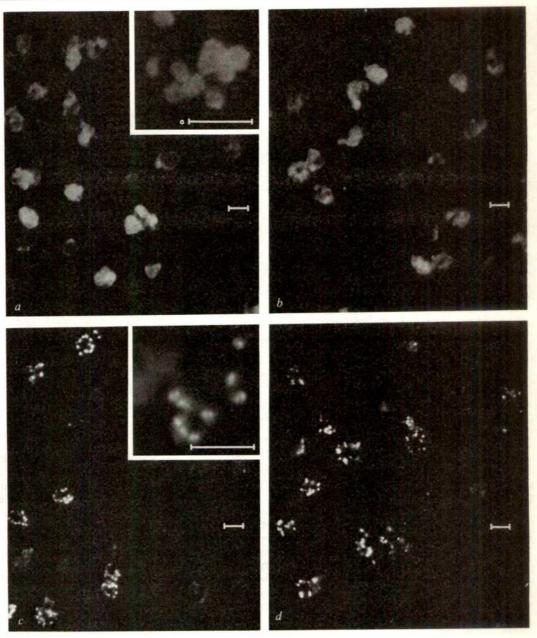


Fig. 2 Processing of the 230,000 MW schizont protein during incubation P. y. yoelii-parasitized erythrocytes in vitro. Parasitized erythrocytes  $(4 \times 10^9)$  were incubated at 37 °C in 8.0 ml of methionine-free RPMI 1640 (Gibco) tissue medium supplemented with 10 µCi ml<sup>-1</sup> 35S-methionine (Amersham). After 10 min, 400 µl of 50 mM methionine was added, and 0.5-ml aliquots of cell suspension were removed at the times indicated. The cells were washed with ice-cold RPMI 1640 and then frozen at -80 °C. The frozen cell pellets were thawed by addition of 0.5 ml of 50 mM Tris-HCl pH 8.0, 5 mM EDTA, 5 mM EGTA, 5 mM iodoacetamide, 1 mM phenylmethyl sulphonyl fluoride, 0.1 mM TLCK, 1% (v/v) NP40, and used for immunoprecipitation using antibody 25.1. After SDS-PAGE on

a 7.5% gel, 35S-labelled polypeptides were detected by fluorography at -70 °C. The estimated MWs of the immunoprecipitated polypeptides are indicated (see Fig. 1). Duration of chase (min); a, 0; b, 10; c, 20; d, 40; e, 60; f, 90; g, 120; h, 150; i, 180; j, 240; k, 300; l, 360; m, 420.

Fig. 3 IIF staining reactions on acetone-fixed P. y. yoelii YM antigen smears, using serum from BALB/c mice carrying the hybridoma lines WIC 25.1 (a) and 25.77 (c), or using serum from mice immunized with the 230,000 MW P. y. yoelii protein and its fragments (b) and the 235,000 MW P. y. yoelii protein (d), as described in Fig. 4 legend. The IIF assay was performed as previously described<sup>1</sup>, using immuno-absorbent-purified fluorescisothiocyanate-labelled rabbit anti-mouse IgG. Inset a, IIF reaction of antibody 25.1 on a mature segmented schizont. Note localization of staining to a membrane surrounding individual merozoites. Inset c, IIF reaction of antibody 25.77 on a mature segmented schizont. Note localization of staining to a small area on each merozoite. Scale bar, 5 µm.



monoclonal antibody. Amino acid sequence homology between the major immunoprecipitated polypeptides was confirmed by two-dimensional chymotryptic peptide mapping (data not shown). In vitro, degradation or processing of the native protein began soon after its synthesis and was extensive after 7 h (Fig. 2). The results indicate that the processing occurred during incubation of the intact schizonts rather than after cell lysis and solubilization. As a consequence of the phenomenon, our purified 25.1 protein preparations always contained fragments of the 230,000 MW protein as well as the intact protein.

In the IIF test, antibody 25.1 reacted with free merozoites in suspension, indicating that the 230,000 MW protein (or fragments of it) is located on the merozoite surface. Using acetone-fixed schizonts, the IIF staining by antibody 25.1 suggests that the 230,000 MW protein is synthesized before merozoite formation and is associated specifically with the plasma membrane of the developing intracellular parasite (Fig. 3a), rather than with the host erythrocyte plasma membrane as suggested previously<sup>2</sup>. In early schizonts the antigen seems to be localized at a spherical membrane enclosing the parasite, but following segmentation and formation of the residual body, the staining is evenly distributed over each merozoite (Fig. 3a, insert).

Antibody 25.77 did not react with free merozoites in suspension in the IIF test, and with acetone-fixed schizonts the reaction was localized to a region within each merozoite (Fig. 3c, insert). It seems, therefore, that the 235,000 MW protein is not on the surface, but may be associated with intracellular organelles of the mature merozoite.

Groups of BALB/c mice were immunized with the two P. y. yoelii protein preparations on three occasions. One week after the third immunization, the mean serum titres were 1 in 10,240 (merozoite-specific) for the group immunized with the 235,000 MW protein, 1 in 10,240 (schizont-specific) for the group immunized with the 230,000 MW protein and its fragments, and 1 in 40 (nonspecific) for the control group.

The sera from the immunized mice immunoprecipitated the same polypeptides as did the monoclonal antibodies (Fig. 1e, f), and produced IIF staining patterns indistinguishable from those produced by the monoclonal antibodies (Fig. 3), confirming the immunogenicity and purity of the parasite protein preparations.

The groups of mice immunized as described were challenged by intravenous (i.v) injection of 10,000 P. y. yoelii YM-parasitised erythrocytes. In the control group, parasitaemia increased rapidly and all mice in this group died on day 8 after challenge (Fig. 4). In contrast, the mice immunized with the 230,000 MW protein and its fragments showed relatively low-grade parasitaemias which were cleared from blood smears by day 10, and all mice in this group survived. In the group immunized with the 235,000 MW protein, parasitaemia was initially suppressed

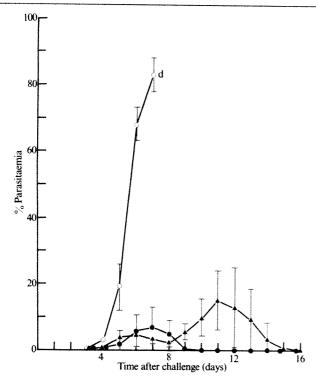


Fig. 4 Mean parasitaemias in groups of five BALB/c mice challenged with 10,000 P. y. yoelii YM-parasitized erythrocytes, after immunization with the P. y. yoelii protein and its fragments (1), or with the 235,000 MW P. y. yoelii protein (\( \begin{array}{c} \) or saline (\( \cappa \)). The P. y. yoelii proteins were purified as described in Fig. 1 legend, and the immunization protocol was as follows: 12 µg of protein in Freund's complete adjuvant (FCA) given intraperitoneally (i.p.) on days 0 and 35, and 20 µg of protein in saline given i.v. on day 50. In the control group, 0.1 ml of saline in FCA was given i.p. on days 0 and 35, and 0.1 ml of saline was given i.v. on day 50. The challenge infection was given i.v. on day 60. Parasitaemia was scored daily by microscopic counting of parasitized erythrocytes in smears of tail blood stained with Giemsa's stain. Mean survival time in the control group is indicated (d). The vertical bars indicate standard deviations

relative to controls, but after day 8 an influx of reticulocytes into the circulation was coincident with an increase in parasitaemia, with all parasites confined to reticulocytes. However, parasitaemias in this group were cleared from blood smears by day 16, and all the mice immunized with the 235,000 MW protein survived.

Immunization with the 230,000 MW schizont antigen and its fragments induced an immune response equally effective against parasites in reticulocytes and in mature erythrocytes. In immunized mice, challenge infection developed normally for the first 3 days, was then suppressed relative to controls, and cleared over the following 7 days. This observation indicates that resistance to infection in these mice was not due simply to the serum antibody response to immunization. The kinetics of challenge infection were similar to those in mice immunized with whole formalin-fixed, free P. y. yoelii parasites described by Playfair et al.3. These workers have implicated both humoral and cellmediated immune responses in the mechanism of parasite clearance in mice immunized with whole parasites<sup>4,5</sup> and it will be important to analyse further the mechanisms mediating immunity in mice immunized with the purified P. y. yoelii proteins.

The ability of P. y. yoelii strains to invade mature erythrocytes is an important factor in their virulence<sup>6</sup>. As had previously been observed<sup>2</sup> after passive transfer of antibodies 25.77 and 25.37. challenge infections in mice immunized with the 235,000 MW merozoite antigen were confined to reticulocytes, and the infections were non-lethal. In unimmunized controls, on the other hand, mature erythrocytes also became extensively parasitized, leading to the deaths of these mice. How the 235,000 MW antigen might be involved in host cell specificity and virulence of P. y. yoelii is not understood.

Other protective P. y. yoelii antigens may exist, and it should be possible, using the approach described here, to identify and characterize them. To extend the present findings, it will be important to determine whether proteins analogous to the 230,000 MW and 235,000 MW antigens of P. y. yoelii are synthesized by other malaria parasites, in particular the human malaria parasite, Plasmodium falciparum.

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#### Complete in vitro maturation of Plasmodium falciparum gametocytes

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The form of malaria caused by Plasmodium falciparum is probably the most important infectious disease of man. In tropical Africa alone, where malaria affects almost the entire population, it has been estimated that every year the disease causes the death of 1 million children under 14 yr old1. The mosquito vector of malaria is infected when it ingests mature gametocytes in blood taken from a human carrier of the disease. Development of the parasite continues in the mosquito and culminates with the sporozoite stage, which initiates a new infection when injected by the mosquito into a further human host. Research on P. falciparum sporozoites has been severely limited by a general lack of availability of suitable patients with gametocytes. It was hoped that the recently developed continuous cultivation of *P. falciparum*<sup>2</sup> would solve this, as gametocytes are often produced during cultivation. However, we and others soon found that these gametocytes failed to mature in culture<sup>3,4</sup>, or at best did so only rarely and unpredictably<sup>5-7</sup>. We report here that addition of hypoxanthine to the culture medium permits the production of mature, infectious P. falciparum gametocytes on a regular basis.

Gametocyte production involves two distinct processes: an inductive process that commits certain asexual parasites to become gametocytes, and a developmental process that supports the full differentiation of these committed cells<sup>3</sup>. Gametocyte induction has been shown to occur more readily when the cultures are maintained for extended periods of time without the addition of fresh uninfected erythrocytes<sup>4,6</sup>. We have found that this induction process is enhanced when the erythrocyte concentration is reduced for part of the culture period, and when old cultures with mature gametocytes are used to initiate new cultures (Table 1). When the erythrocyte concentration was reduced to 6%, the resulting gametocytaemia was almost double that observed for an erythrocyte concentration of 12%. Interestingly, the absolute numbers of gametocytes per culture flask remained about the same in both cases.

Gametocytes grown in these conditions, and with added hypoxanthine (50 μg ml<sup>-1</sup>), developed to functional maturity (as determined by their infectivity for mosquitoes (Tables 2-4)). When the gametocytes were collected 12-14 days after initiation of the cultures, mosquitoes in each of 18 groups that fed on

Table 1 Effect of erythrocyte concentration on production in vitro of Plasmodium falciparum gametocytes

Strain	Erythrocyte concentration	No. of cultures	Gametocytaemia*
Honduras	12%	72	$1.5 \pm 0.5\%$
110	6%	74	$2.7 \pm 0.5\%$
Z	12%	23	$1.4 \pm 0.6\%$
	6%	24	$2.9 \pm 0.6\%$
FCN-2	12%	28	$1.0 \pm 0.5\%$
	6%	26	$2.3 \pm 0.6\%$

All cultures were initiated at 0.2% parasitaemia and 12% haematocrit in RPMI-1640 with HEPES<sup>2</sup>, 15% (v/v) human serum and 50 µg ml<sup>-1</sup> hypoxanthine (Sigma). Human erythrocytes were used within a few days after collection. Cultures were grown at 37 °C in tissue culture flasks (75 cm<sup>2</sup>) in a total volume of 20 ml per flask, in an atmosphere of 3% O<sub>2</sub>, 2% CO<sub>2</sub> and 95% N (ref. 15). Medium was changed daily by removal of spent medium and addition of fresh medium prewarmed to 37 °C. No new erythrocytes were added during the culture period. At 7 days the haematocrit of some cultures was reduced to 6%, and daily feeding of all cultures continued with the same medium, but with the human serum component reduced to 10%. Gametocytaemia was determined at 12-14 days, and these mature cultures were used to initiate new gametocyte cultures. The Honduras strain was from CDC, Z was obtained from NIH and FCN-2 was isolated from blood of a patient from Bangkok.

\* Number of gametocytes (±s.d.) per 100 erythrocytes, determined from Giemsa-stained smears.

them developed midgut infections consisting of substantial numbers of oocysts. In each of these groups, the percentage of mosquitoes that went on to develop salivary gland infections of sporozoites was comparable to the percentage with oocyst infections. Sporozoites collected from the salivary glands appeared normal, and had motility typical of *P. falciparum* sporozoites<sup>8</sup>.

Complete gametocyte maturation did not normally occur in parallel control cultures without added hypoxanthine (Table 2). Gametocytes in these control cultures appeared morphologically normal, but were generally not infective for mosquitoes. In a single case, a mosquito fed on gametocytes from these control cultures was found to have one oocyst in its midgut.

Asexual, erythrocytic malarial parasites are known to require exogenous sources of purines for their metabolism, but rely on de novo synthesis of pyrimidines<sup>9</sup>. Studies of the sporogenic

Table 2 Effects of hypoxanthine on gametocyte maturation in infected vectors

	No. of mosquitoes infected with oocysts					
Expt	Hypoxanthine (50 µg ml <sup>-1</sup> )	No hypoxanthine				
1	11/17 (65%)	0/15 (0%)				
2	8/20 (40%)	0/20 (0%)				
3	14/18 (78%)	1/17* (6%)				
4	13/20 (65%)	0/13 (0%)				
5	10/16 (63%)	0/15 (0%)				

Gametocytes (Honduras strain) were cultured as described in Table 1 legend, with or without hypoxanthine added to the culture medium, and collected 13-14 days after initiation of the cultures. Cells in the culture flask were sedimented, after which 2-3 vol of uninfected human erythrocytes were added together with sufficient human serum to give a 50% haematocrit, and fed to Anopheles stephensi mosquitoes using a membrane feeder 16. Mosquitoes were maintained according to standard procedures 17 and received a blood meal from an uninfected hamster on day 6. Midgut infections were determined 10-14 days after the infective feeding. Values shown are the results of individual experiments.

\* Infection with single oocyst.

Table 3 Infectivity of gametocytes collected at different times after initiation of cultures

Age of gametocytes fed to mosquitoes (no. of days			No. of mo	th oocysts		
in culture)	Ho	nduras st	strain FCN-2 strain		n	
11	6/14 (43%)					
12	8/14 (57%)	8/8 (100%)	5/12 (42%)	7/8 (88%)		
13	4/12 (33%)			6/10 (60%)		
14	20/20 (100%)	25/27 (93%)	11/22 (50%)	10/24 (42%)	25/27 (83%)	4/12 (33%)
15	30/56 (54%)	0/15 (0%)	4/22 (18%)		8/18 (44%)	
16			0/9 (0%)			
19	14/28 (50%)	0/30 (0%)		8/15 (53%)		

Gametocytes were cultured with added hypoxanthine and fed to mosquitoes as described in legends to Tables 1 and 2. Midgut infections were determined 10-14 days after the infective feeding. Values shown are the results of individual experiments.

stages of the parasite suggest that a similar situation may hold for ookinetes<sup>10</sup> and sporozoites<sup>11</sup>. There is evidence<sup>9</sup> that the key exogenous purine incorporated by the parasite is hypoxanthine. We have previously pointed out<sup>3</sup> that RPMI-1640 is a relatively simple tissue culture medium that supplies all nutritional requirements for asexual parasites but that gametocytes may require additional growth factors for full development. For successful cultivation of asexual parasites, the host erythrocyte is obliged to supply nutrients for only the 48-cycle of the parasite's development within it, whereas the developing gametocyte occupies its host erythrocyte for 2 weeks or longer. For complete gametocyte maturation to occur, the depleted hypoxanthine reserves of the host erythrocyte apparently must be supplemented by an exogenous source. Our finding that hypoxanthine may be a limiting factor in the culture of P. falciparum gametocytes should now permit large-scale production of mature gametocytes for further immunization studies using gametes<sup>12</sup> or sporozoites<sup>13,14</sup> of this species.

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 Table 4
 Oocyst production in Anopheles stephensi mosquitoes fed on Plasmodium falciparum gametocytes grown in vitro

Age of gametocytes fed to mosquitoes (no. of days in culture)	Ho		er infect	. of oocyst ed mosqui F6		n
11	2.8			,0112A041110000111000011100001110000111100001111		
12	3.3	7.9	4.8	102.5	120.1	
13	2.0			3.0		
14	64.0	47.0	9.0	6.5	34.0	1.5
15	31.6		3.0	5.5	25.4	2.0
19	4.5			5.0		

Gametocytes were cultured with added hypoxanthine and fed to mosquitoes as described in Tables 1 and 2. Oocysts were counted 10-12 days after the infective feeding. Values shown are the results of individual experiments.

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#### Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum

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Two types of dopamine receptor whose stimulation affects cellular cyclic AMP have now been identified. In tissues as different as insect ganglia and mammalian neostriatum, stimulation of the dopamine receptor called D-1 increases formation of cyclic AMP<sup>1-6</sup>, whereas stimulation of the second type of dopamine receptor (D-2)<sup>7-11</sup>, first identified in the rat pituitary gland, reduces cyclic AMP formation. Recently, a receptor with the pharmacological properties of the D-2 receptor has also been found 12-14 in the rat neostriatum; we show here that stimulation of this receptor is followed by a reduction in cyclic AMP formation induced by V stimulation with D-1 agonists.

Previous investigations of the dopaminergic regulation of neostriatal cyclic AMP formation have determined either adenylate cyclase activity of tissue homogenates<sup>2</sup> or cyclic AMP content of tissue slices<sup>15</sup>. However, we have used as a biochemical sign of dopamine receptor stimulation the dopaminergic enhancement of cyclic AMP efflux from slices of neostriatum because cyclic AMP efflux is a particularly sensitive indicator of hormone-stimulated cyclic AMP formation in the adrenal cortex<sup>16,17</sup>. Blocks of rat neostriatum were superfused with Earle's balanced salt solution, to which were added isobutyl methylxanthine<sup>18</sup> (1 mM) and bovine serum albumin (BSA; 2.5 mg ml<sup>-1</sup>) in a 16-chamber superfusion apparatus described elsewhere 19. After 60 min of superfusion, several fractions were collected for each chamber, and dopaminergic drugs were added to the medium and additional fractions collected. At the end of the experiment, the cyclic AMP content of each fraction was determined by radioimmunoassay.

SKF 38393 is an agonist equipotent (on a molar basis) to dopamine on the D-1 dopamine receptor 4,20,21, but substantially less potent (on a molar basis) when tested on the pituitary D-2 dopamine receptor 11,20. SKF 38393 caused a time- and dosedependent increase in the efflux of cyclic AMP from blocks of rat neostriatum (Fig. 1a, b), the maximal effect being a 3.5-fold enhancement of cyclic AMP efflux which occurred after 25 min. This maximal effect on cyclic AMP efflux was greater than the maximal effect of the agonist on either the cyclic AMP content of neostriatal blocks (1.6-fold enhancement; see Fig. 1 legend) or

Table 1 Fluphenazine inhibits SKF 38393-stimulated efflux of cyclic

Drug	Cyclic AMP efflux (% of basal efflux)
None	$99.8 \pm 12.7$
SKF 38393 (1 μM)	$304.0 \pm 18.5$
SKF 38393 $(1 \mu M)$ + fluphenazine $(1 \mu M)^*$	$194.3 \pm 12.3 \dagger$
SKF 38393 $(1 \mu M)$ + fluphenazine $(10 \mu M)^*$	$121.8 \pm 2.4 \dagger$

Experimental procedures were as described for Fig. 1a, Basal efflux of cyclic AMP was determined for three successive 10-min fractions collected between 60 and 90 min. Exposure to drugs began at 90 min. Cyclic AMP efflux in the final fraction (115-125 min) was expressed as mean % of basal efflux (±s.e.m.) of observations made on four superfusion chambers (as described for Fig. 1b).

Fluphenazine was added 30 min before SKF 38393 (see ref. 29).

on the adenylate cyclase activity (1.7-fold enhancement<sup>20</sup>). SKF 38393 half-maximally enhanced cyclic AMP efflux at a concentration of  $2 \times 10^{-7}$  M (Fig. 1c), which is about the same as the concentration (10<sup>-7</sup> M) reported half-maximally to activate neostriatal adenylate cyclase activity<sup>20</sup>. Fluphenazine markedly attenuated the SKF 38393-stimulated efflux of cyclic AMP (Table 1). However (-)sulpiride, an antagonist on the pituitary D-2 dopamine receptor<sup>11,22</sup> which has no antagonist action on the D-1 receptor<sup>23</sup>, did not alter the basal efflux of cyclic AMP and induced a statistically insignificant potentiation of the SKF 38393-stimulated efflux (Fig. 2a). These results demonstrate that the efflux of cyclic AMP from blocks of rat neostriatum provides a biochemical sign of stimulation of the D-1 receptor in this tissue.

LY-141865, an agonist on the pituitary D-2 receptor<sup>24,25</sup> which has no effect on the D-1 receptor in fish retina25 or rat neostriatum (E. Frey, unpublished results), did not stimulate the efflux of cyclic AMP either in the presence or absence of (-)sulpiride (Fig. 2b). Dopamine, an agonist on both the D-1 (refs 1-6) and D-2 (refs 11, 26, 27) dopamine receptors, increased the efflux of cyclic AMP from blocks of rat neostriatum (Fig. 2c). Interestingly, (-)sulpiride markedly potentiated the dopamine-stimulated efflux of cyclic AMP whereas (+)sulpiride was ineffective (Fig. 2c). Furthermore, apomorphine, which also stimulates both dopamine receptors<sup>1-4,11,26,27</sup>, enhanced the efflux of cyclic AMP only in the presence of (-)sulpiride (Fig. 2d).

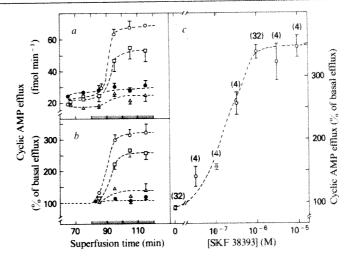
LY-141865 reduced the SKF 38393-stimulated efflux of cyclic AMP, the maximal effect being a reduction of  $57 \pm 8\%$ (data from four separate experiments; Fig. 3). This inhibitory effect was reversed by ( – )sulpiride (Fig. 3). LY-141865 did not significantly inhibit the (-)isoprenaline-stimulated efflux of cyclic AMP: cyclic AMP efflux induced by (-)isoprenaline  $(1 \mu M)$  was  $514.3 \pm 33.8$  (% of basal efflux) compared with  $452.0 \pm 28.3\%$ for (-)isoprenaline  $(1 \mu M) + LY-141865$  $(1 \mu M)$ .

Based on the results obtained with dopamine, apomorphine and LY-141865, we propose the existence in the neostriatum of a second dopamine receptor, regulating the efflux (and by inference the formation) of cyclic AMP. Stimulation of this second receptor inhibits the formation of cyclic AMP occurring as a consequence of stimulating the D-1 dopamine receptor. According to this interpretation, dopamine interacts with both types of receptor in the neostriatum, simultaneously stimulating and inhibiting the formation and efflux of cyclic AMP. (-)Sulpiride potentiates the dopamine-stimulated efflux by blocking the second dopamine receptor, removing the inhibitory constraint on the formation of cyclic AMP and thereby stimulating the observed efflux of cyclic AMP. Similarly, apomorphine stimulates both dopamine receptors; (-)sulpiride antagonizes the interaction between apomorphine and the second dopamine receptor, thereby permitting the interaction between apomorphine and the D-1 receptor to be expressed as

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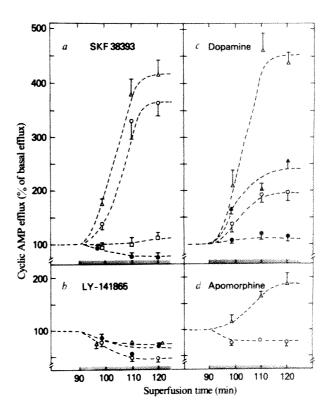
<sup>†</sup> P < 0.001 compared with SKF 38393 alone (Student's t-test).

Fig. 1 SKF 38393 stimulates efflux of cyclic AMP from blocks of rat neostriatum. a, Time course of cyclic AMP efflux. Tissue was superfused without drug ( $\bullet$ ) or with SKF 38393 ( $\triangle$ ,  $3 \times 10^{-8}$  M;  $\square$ ,  $3 \times 10^{-7}$  M;  $\bigcirc$ ,  $3 \times 10^{-8}$  M). Exposure to SKF 38393 (thickened abscissa) began at 80 min. Data represent mean  $\pm$  s.e.m. (n = 4) of the cyclic AMP content of superfusion medium collected during the indicated time periods. At the end of the experiment, tissue content of cyclic AMP was 312 ± 14 fmol per mg wet weight in the absence of SKF 38393 and  $352\pm6$ ,  $428\pm22$  and  $506\pm32$  fmol per mg wet weight in the presence of  $3\times10^{-8}\,\mathrm{M}$ ,  $3\times10^{-7}\,\mathrm{M}$  and  $3\times10^{-6}\,\mathrm{M}$  SKF 38393 respectively. In all experiments, brains of eight male Sprague–Dawley rats  $(250-300\,\mathrm{g})$  were removed and placed in a special holder. Two successive 2 mm-thick coronal brain slices were made between the anterior planes A 7,000 and A11,000 µm (ref. 30). Neostriatal tissue was dissected from these two slices and cut twice with a McIlwain tissue chopper (300 μ); before the second pass, the chopping surface was rotated 90°. The resulting blocks of neostriatal tissue (2 × 0.3 × 0.3 mm) were transferred to Earle's balanced salt solution (EBSS) and stored until neostriatal tissue had been obtained from all rats. Aliquots



(20 mg wet weight) of the chopped neostriatal tissue were placed in each of 16 chambers of a superfusion apparatus<sup>19</sup> and superfused at a rate of 0.1 ml min<sup>-1</sup> with EBSS (37 °C) containing BSA (2.5 mg ml<sup>-1</sup>) and 3-isobutyl-1-methylxanthine<sup>18</sup> (1 mM). After 60 min of superfusion, two or three 1.0-ml fractions (as indicated in the legends) were collected from each chamber; drugs were added to the superfusion medium and additional fractions collected from each chamber. Although no antioxidant was included during the superfusion, decomposition (as determined by HPLC with electrochemical detection) of drugs known to be sensitive to oxidation, such as dopamine, (-)isoprenaline or apomorphine, was <10% during the 30 min of superfusion. Each data point on the ordinate represents the midpoint of the collection period (for example, the data obtained from fractions collected between 80 and 90 min are located at a superfusion time of 85 min). At the end of the experiment, the tissue was superfused for 20 min with 0.1 M HCl to extract cyclic AMP from the tissue. The cyclic AMP content of duplicate 100-µl aliquots of each fraction was estimated by radioimmunoassay (RIA) as described elsewhere<sup>31</sup> using RIA kits (NEN); the limit of detection of this RIA was 2.5 fmol per 100 µl. b, Data from a transformed mathematically. The ability of SKF 38393 to enhance cyclic AMP efflux was calculated from data in a (the same symbols are used). Every chamber served as its own control; for each chamber, the basal efflux of cyclic AMP (that is, the mean amount of cyclic AMP in the fractions collected before drug addition) was calculated, and the amount of cyclic AMP determined from data obtained in nine separate experiments; the SKF 38393-induced stimulation of cyclic AMP efflux was calculated as in b. The data represent SKF 38393-stimulated cyclic AMP efflux in the last collected fraction (for example, the fraction collected between 110 and 120 min in a). Values represent mean ± s.e.m.; n, indicated in parentheses, is the number of supe

Fig. 2 Effects of dopaminergic agonists and sulpiride on efflux of cyclic AMP from blocks of rat neostriatum. Experimental procedures were as described for Fig. 1a. Basal efflux of cyclic AMP was determined from three successive 10-min fractions collected between 60 and 90 min. Exposure to drugs (thickened abscissa) began at 90 min. Cyclic AMP efflux was expressed as the mean % basal efflux (±s.e.m.) of observations made on four superfusion chambers (as described for Fig. 1b). a, Tissue was superfused without drug (●), or with 10<sup>-6</sup> M (−) sulpiride (□), 10<sup>-6</sup> M SKF 38393 (○) or 10<sup>-6</sup> M (−) sulpiride and 10<sup>-6</sup> M SKF 38393 (△). b, Tissue was superfused without drug (●) or with 10<sup>-6</sup> M LY-141865 (○) or 10<sup>-6</sup> M LY-141865 and 10<sup>-6</sup> M (−) sulpiride (△). (LY-141865 is designated 'compound 38, R = pro' in ref. 24.) c, Tissue was superfused without drug (●) or with 8×10<sup>-6</sup> M dopamine (○), 8×10<sup>-6</sup> M dopamine and 2×10<sup>-6</sup> M (−) sulpiride (△), or 8×10<sup>-6</sup> M dopamine and 2×10<sup>-6</sup> M (+) sulpiride (▲). d, Tissue was superfused with 5×10<sup>-7</sup> M apomorphine alone (○) or in combination with 10<sup>-6</sup> M (−) sulpiride (△).



an enhanced efflux of cyclic AMP (although apomorphine can also antagonize the D-1 receptor  $^{3,21}$ , our experiments were not designed to demonstrate this property). The SKF 38393-stimulated efflux of cyclic AMP was not significantly potentiated by (—)sulpiride because, at a concentration of 1  $\mu$ M, SKF 38393 stimulates the D-1 dopamine receptor without having a significant effect on the D-2 receptor. However, the SKF 38393-

stimulated efflux of cyclic AMP was diminished by LY-141865 as a consequence of the interaction between LY-141865 and the second, pituitary-like D-2 dopamine receptor. Accordingly, this effect of LY-141865 was blocked by (-)sulpiride.

In the intermediate lobe of the rat pituitary gland, a  $\beta$ -adrenoceptor and a D-2 dopamine receptor occur on the same cell. Stimulation of the D-2 dopamine receptor decreases the

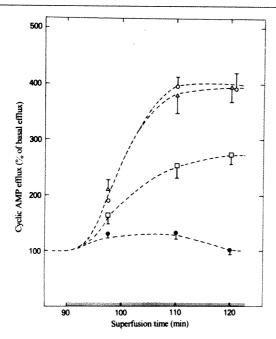


Fig. 3 Inhibitory effect of LY-141865 on SKF 38393-stimulated efflux of cyclic AMP is antagonized by (-)sulpiride. Experimental procedures were as described for Fig. 1a. Basal efflux of cyclic AMP was determined from three successive 10-min fractions collected between 60 and 90 min. Exposure to drugs (thickened abscissa) began at 90 min. Efflux of cyclic AMP was expressed as the mean % basal efflux (±s.e.m.) of observations made on four superfusion chambers (as described for Fig. 1b). Tissue was superfused without drug (●), or with 10<sup>-6</sup> M SKF 38393 (○), 10<sup>-6</sup> M SKF 38393 and 10<sup>-6</sup> M LY-141865 (□), or with 10<sup>-6</sup> M SKF 38393, 10<sup>-6</sup> M LY-141865 and 10<sup>-6</sup> M (−)sulpiride (△).

formation of cyclic AMP stimulated by  $\beta$ -adrenergic agonists. By analogy with the situation in the intermediate lobe, the biphasic regulation of cyclic AMP formation by dopamine raises the possibility that both the D-1 and D-2 dopamine receptors are associated with the same cell in the neostriatum. However, it is possible that stimulation of the D-2 receptor decreases the responsiveness of the D-1 receptor by an indirect, trans-synaptic process. Although a  $\beta$ -adrenoceptor enhances cyclic AMP formation in the neostriatum<sup>15,28</sup>, the failure of LY-141865 to affect significantly the (-)isoprenaline-stimulated efflux of cyclic AMP suggests that the D-2 dopamine receptor and the  $\beta$ -adrenoceptor are not functionally connected in the neostriatum. Although our data demonstrate a biochemical consequence of stimulation of the D-2 dopamine receptor in the neostriatum, the ultimate physiological consequences of stimulation of either the D-1 or the D-2 receptor in this area of the brain remain unknown.

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#### Single channel potassium currents of the anomalous rectifier

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The recording of single channel currents has revealed the discrete events underlying the conductance changes for the Na and delayed K currents of the action potential, as well as those underlying acetylcholine-evoked currents<sup>1-5</sup>. The existence of similar discrete events has been suggested by noise analysis of the current termed anomalous K rectifier<sup>6,7</sup>, in which a K conductance is increased by hyperpolarization or by an increased extracellular concentration of K ions8. I have now used the patch current recording technique to investigate the anomalous rectifier of the tunicate egg cell. The statistics of pulse-like events in the patch current were found to be consistent with the inactivation kinetics of the anomalous rectifier. The extrapolated zero-current potential of the pulse-like events was approximately equal to that of the total anomalous K current. The single channel conductance was 5.2 pS in 50 mM K solution.

Tunicate egg cells were voltage-clamped with two microelectrodes as described previously9. The bath saline contained 200 mM NaCl, 360 mM choline chloride and 10 mM MnCl<sub>2</sub>, pH 7.0, and was maintained at 13.5-15.5 °C. A clean membrane surface was obtained by enzymatic digestion of the chorion. Patch current was recorded with a current-voltage converter which had a feedback resistor of 500 M $\Omega$ . The patch electrode was a conventional micropipette that had not been fire polished, and was filled with saline similar to that in the bath, except that 50, 100 or 200 mM K replaced an equal amount of choline. CaCl<sub>2</sub> (10 mM) and MgCl<sub>2</sub> (30 mM) were used as divalent salts; later 10 mM MnCl<sub>2</sub> was found to provide more stable recordings. The resistance of the patch pipette was  $10-50 \text{ M}\Omega$ . When pushed against the egg surface, the seal resistance increased to >1 G $\Omega$  (usually 5-8 G $\Omega$ ). The background noise level was 0.4-0.8 pA (peak-to-peak value, low-pass filtered at 660 Hz with a three-pole Butterworth filter). The rise time between 10 and 90% of the current step recorded by the patch electrode was 0.7-1.0 ms. Current records were digitized at 200 µs per point and stored by an 8-bit, 1K-word transient recorder (Kawasaki Electronica TM 1510).

After obtaining a large seal resistance, 150-ms hyperpolarizing pulses of up to -200 mV were applied to the whole egg cell, from a holding potential of -10 mV. Pulse-like changes in the patch current were observed which had a uniform amplitude at a given membrane potential (Fig. 1c). The pulse-like events in a patch usually disappeared within 20 command pulses. In some such cases, the amplitude of the pulse-like events was reduced in size during the last two or three command pulses and the traces for these were discarded. The current traces at -170 mV in

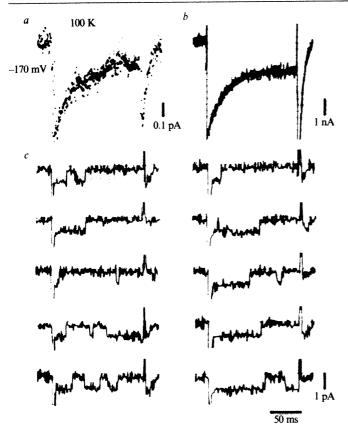


Fig. 1 a, Averaged time course of the pulse-like events in a patch. The whole egg cell was hyperpolarized to  $-170\,\mathrm{mV}$  during 45 command pulses. K concentration in the patch pipette was 100 mM, indicated as 100 K in this and other figures. b, Total membrane K current of the same egg cell in the same solution as in the patch pipette. The estimated number of channels was 7,200 per egg. c, Ten representative traces from the averaged 45 current traces. The inward current after the command pulse in a, b and c was Na current, which was evoked at the holding level of  $-10\,\mathrm{mV}$  after the hyperpolarizing command pulses relieved the inactivation of Na current.

100 mM K solution mostly started with inward deflections after the 3-6-ms capacitative transient and showed two to three pulse-like changes in the patch current during the command pulse lasting 150 ms. Multiples of the unitary amplitude were observed only exceptionally (Fig. 2b), indicating that, in most cases, a single channel existed under the patch pipette. Only the current traces from patches with one channel were analysed. Before or after patch recording the solution in the experimental bath was replaced by that contained in the patch pipette and a conventional voltage-clamp experiment was performed to examine the total anomalous K current. With a hyperpolarizing pulse of -170 mV the total anomalous K current showed an almost instantaneous rise and a subsequent slow exponential decay due to inactivation (Fig. 1b). When the pulse-like events in the patch current obtained at the same potential level were averaged, the time course was similar to that of the total anomalous K current (Fig. 1a). When the command pulse potential was less negative than -170 mV, the amplitude of the pulse-like events became smaller and the number of the events in a current trace decreased. Few or no events were observed at potentials more positive than -130 mV (Fig. 2a, -130 mV).

To analyse the kinetics of the pulse-like events in the patch current, the duration of the first open events was measured and it was denoted as the open time. The closed time was defined as the duration of the lower conductance period following the first open event (Fig. 3); unless it was terminated by another opening, the closed period was not counted. The distribution of the open or closed time showed a monotonic decay with duration; the distribution of the open time at -170 mV was roughly

exponential. The mean open time at  $-130\,\mathrm{mV}$  was markedly longer than at  $-170\,\mathrm{mV}$ , while the mean closed time at  $-130\,\mathrm{mV}$  was slightly shorter than at  $-170\,\mathrm{mV}$ . Assuming that the inactivation process can be described as a first-order transition between the open and closed states,

open state 
$$\stackrel{\alpha}{\underset{\beta}{\longleftarrow}}$$
 closed state

and that all channels are open immediately after the start of the command pulse, the probability density functions for the open time,  $f_{\rm open}(t)$ , and the closed time,  $f_{\rm closed}(t)$ , are

$$f_{\text{open}}(t) = \beta \exp(-\beta t)$$
  
$$f_{\text{closed}}(t) = \alpha \exp(-\alpha t)[1 - \exp\{-\beta (T - t)\}]$$

T is the duration of the command pulse, here equal to 150 ms. The expressions are derived as follows. The probability that a channel remains open at a time t following its opening is  $p = \exp(-\beta t)$ , so the probability density function describing the probability that it closes exactly at t—that its lifetime is t—is

$$f_{\text{open}}(t) = -dp/dt = \beta \exp(-\beta t)$$

A similar expression would describe the probability density function for the closed times, except that the termination of the observation period T by the end of the command pulse reduces the probability of observing long closed times. The reduction depends on the distribution of the open times:

$$f_{\text{closed}}(t) = \alpha \exp(-\alpha t) \int_0^{T-t} \beta \exp(-\beta t') dt'$$
$$= \alpha \exp(-\alpha t) [1 - \exp\{-\beta (T-t)\}]$$

The closing rate constants  $\beta$ , which were estimated from the total anomalous K currents, were 4.01 and  $18.97~{\rm s}^{-1}$ , and the opening rate constants  $\alpha$  12.78 and  $9.46~{\rm s}^{-1}$ , at -130 and  $-170~{\rm mV}$  respectively. In Fig. 3, the probability density functions for single channels estimated from these rate constants are superimposed on the actual distribution by solid thick lines. The actual distributions were in good agreement with the estimated curves; deviations may be due to the small sample

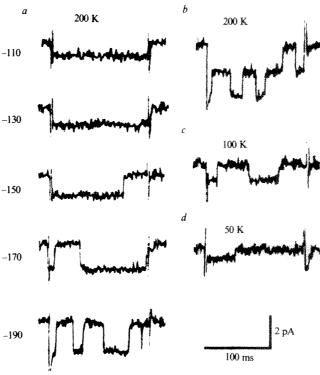


Fig. 2 a, Patch current in 200 mM K solution (200 K). The potential levels (in mV) during the command pulses are shown on the left. b, Current through a patch with two channels in 200 mM K solution. c, d, Patch current in 100 and 50 mM K solutions. The potential levels were  $-170 \,\mathrm{mW}$  in b, c and d.

numbers. The asymmetric dependencies of the opening and closing rate constants on the membrane potential, which were observed from the decay time course of the total anomalous K current, were confirmed in the kinetics of a single channel. It was concluded that the statistics of single channel events in the patch current reflect the inactivation process of the anomalous K rectifier.

The amplitude of the pulse-like events in individual patches was constant at a given membrane potential, with some variations due to the background noise (Fig. 4a). The amplitude of the pulse-like events in a patch was linearly related to the membrane potential in the range -200 to -100 mV (Fig. 4b). Zero-current potentials of the pulse-like events were estimated by extrapolating the least-squares fits to the amplitudes for those patches from which current traces were recorded at least at three membrane potentials. The values of the estimated zero-current

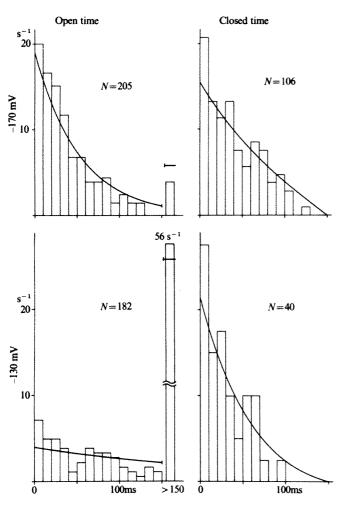


Fig. 3 The distribution of open and closed times, plotted as probability density. The patch current was recorded in 100 mM K solution. Only the first open and closed events of each record were measured, because later events were suspended by the end of the command pulse and accurate measurement of the durations was impossible. Cases in which the channel remained open during the 150-ms command pulse were plotted at the right edge in the distribution of the open times. Cases in which the channel failed to reopen during the command pulse are not included in the distribution of closed times. In the cases where the channel started in the closed state after the artefact, the open event was considered to have terminated during the artefact and was plotted in the first column in the distribution of the open time. The closed time in these cases was calculated by using an open time calculated from the duration of the artefact and the predicted distribution curve. N in each plot is the total sample number, which was normalized to 1 for comparison with the calculated probability density functions. The probability density function for the closed time was normalized by the integral from zero time to 150 ms.

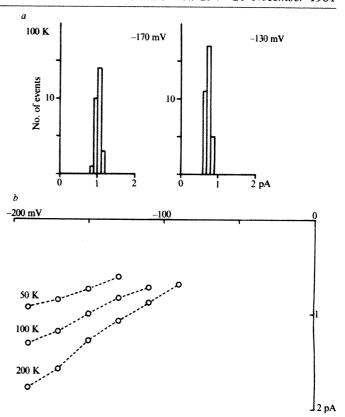


Fig. 4 a, Amplitude distribution of the pulse-like events in a single patch. The membrane potential was -170 and -130 mV in 100 mM K solution. b, Membrane potential and the amplitude of the pulse-like events in a patch. The K concentration in the patch pipette is indicated for each curve. The mean amplitude was plotted for several current traces recorded at a certain potential level.

potentials were  $-35\pm7$ ,  $-30\pm19$  and  $-24\pm14$  mV (mean  $\pm$  s.d.) in 50, 100 and 200 mM K solutions respectively. The zero-current potentials of the total anomalous K current were measured by the resting potentials in Na-free 50, 100 and 200 mM K solutions, giving values of -37, -20 and -3 mV respectively. The difference in the zero-current potentials between those extrapolated from the amplitude of the pulse-like events in the patch current and those measured with the total anomalous K current may be due to a nonlinear current-voltage relationship for single channels.

At -170 mV in 50 mM K solution, the mean amplitude of the pulse-like events occurring in different patches was 0.69 ± 0.14 pA and the single channel conductance was  $5.2 \pm 1.0$  pS, using a zero-current potential of the total anomalous K current. In 100 and 200 mM K solutions, the mean amplitude of the pulse-like events occurring in different patches showed more scattered distribution than that expected from the background noise. Because the statistics of the pulse-like events did not differ between patches, a heterogeneity of channel types is unlikely. Differences in the seal resistance bore no relation to the scattering of the amplitudes. The smaller amplitude of the events may be attributed to the channels under the patch pipette, as proposed in the case of the acetylcholine current<sup>1</sup> The number of channels in an egg was estimated as the ratio of the amplitude of the total anomalous K current in 50 mM K solution at the start of the command pulse to the single channel current of 0.69 pA at -170 mV, where the activation process was almost instantaneously completed and all channels were considered to be initially open. The estimated value was 9,300 ± 6,000 per egg  $(0.041 \ \mu \text{m}^{-2})$ .

Discrete conductance events have been reported in the delayed and Ca-dependent K current<sup>2,10,11</sup>. My experiment shows that single conductance events also underlie the

anomalous K rectifier. The statistics of single channel events are concluded to be derived from the inactivation of the anomalous rectifier because the distribution of the open and closed times coincided with those estimated from the decay time course of the total anomalous K current. The single channel conductances for the anomalous rectifier estimated by the steady-state and ensemble noise analysis were 5.97 and 5.50 pS in 50 mM K . These values are in good agreement with the value of 5.2 pS in 50 mM K solution obtained in the present experiment. Recently the single channel current of the anomalous rectifier has been recorded in rat myotubes and conductance of similar order was obtained12

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#### Gramicidin forms multi-state rectifying channels

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Gramicidin A is a pentadecapeptide of alternating L and D amino acids1. In membranes it forms cation conductive channels<sup>2</sup> of molecular dimensions<sup>3,4</sup> that in many respects resemble<sup>2-7</sup> the channels of excitable cells<sup>8,9</sup>. For this reason, and also because its structure is well established 1,10,11, the gramicidin channel is regarded as a useful model of ion channels in cell membranes. Although the properties of the gramicidin channel have been studied extensively<sup>12</sup>, it has generally been described as having a single state associated with a sharply defined conductance that remains unaltered during the lifetime of the channel<sup>13</sup>. We report here that, in fact, gramicidin A can assume other, less conductive 'miniature' (mini) states evidenced by our observations of spontaneous transitions in the conductance of single open channels and the observation of a significant number of weakly conducting channels. Current-voltage (I-V) relationships for different channel states differ significantly and, for minis, are often asymmetrical. Our results indicate that the gramicidin channel has a wide variety of stable conformational states that give rise to channels with different electrical properties. Because transitions between states occur relatively infrequently, these conformational states must be separated by relatively large energies of interconversion. Similar transitions, poised by the electric field or an agonist molecule, may underlie the function of gated channels in cell membranes.

Gramicidin A was purified by HPLC from commercially available gramicidin D (a mixture 14 of gramicidins A, B and C). Membrane currents in the presence of small amounts of gramicidin A were amplified and sampled digitally for computerassisted analysis. For channel conductance measurement (Fig. 1), the electrical potential across the membrane was fixed so that the ionic current through single channels could be monitored directly. For single-channel I-V measurements (Fig. 2), the

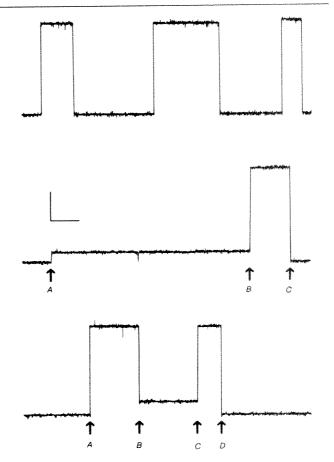


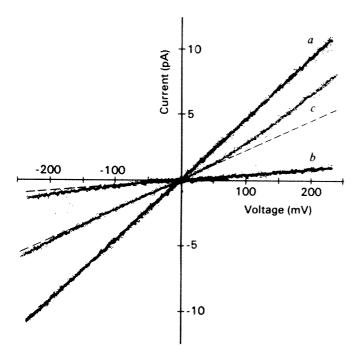
Fig. 1 Time course of the ionic current through single gramicidin A channels. HPLC-purified gramicidin A was added to a bilayer formed from a dispersion of 1-monoolein (50 mg, Nu-Check) in squalene (1 ml, Sigma, purified through silica) and held at 0.1 V. Vertical calibration bar, 1.5 pA. Horizontal bar, 35 s (upper), 10 s (middle) and 15 s (lower). The signal was bandlimited to 30 Hz and sampled 30 times per s. 1M KCL, pH  $\sim$ 5.6, 23  $\pm$  1 °C.

membrane potential was varied at a constant rate, Direct recording of single-channel I-V curves was made possible by reducing the membrane area  $(1.5 \times 10^{-5} \text{ cm}^2)$  so that capacitative current would not obscure the single-channel current.

Channels formed by purified gramicidin A turn on abruptly, that is, with a time course (<200 µs) that cannot be experimentally resolved. The channels typically remain conductive in a state that has a uniform conductance,  $47.5 \pm 0.5 \text{ pS} \pm \text{s.d.}$ , in the conditions of Fig. 1, and cease conducting abruptly and completely<sup>15</sup>. Three examples of these 'typical' channels are shown in the upper trace of Fig. 1. By recording the occurrence of many single channels, we have been able to observe a small but well defined fraction of channels that have novel, atypical properties. The middle trace of Fig. 1 shows, for example, a weakly conducting 'mini' channel (4.6 pS) turning on at point A. At point B the mini channel abruptly becomes more conductive, with a conductance (47.9 pS) characteristic of typical channels, and at point C this channel ceases to conduct. The lower trace of Fig. 1 illustrates that channel conductance may also spontaneously decrease. On this trace a mini channel of high conductance  $(45.2 \,\mathrm{pS})$  turns on at point A, at point B the conductance decreases abruptly and spontaneously to 6 pS and after returning to 45.2 pS at C, the channel turns off at D. We considered whether such traces could merely be the coincidental occurrence of two transitions (such as a turn-on and a turn-off) in the same sampling interval. By using very thin mono-olein/squalene bilayers<sup>15</sup> (and thus maximizing the singlechannel lifetimes), applying only small amounts of gramicidin and sampling rapidly compared with the transition rate, we were able to minimize the possibility of two transitions occurring in the same sampling interval. Twenty-six spontaneous state changes were observed in a contiguous, prospectively studied set of data lasting  $8.25\ h$  ( $8.9\times10^5$  sampling intervals) and containing 424 channels. In our conditions of measurement, the probability that all 26 events resulted from coincidental transitions is <1 in  $10^{41}$ . Therefore we must be observing conductance changes in the same channel. Clearly the same gramicidin channel can assume multiple conductance states.

The diminished conductance of the mini state must arise from a spontaneously increased barrier to ion transport or increased binding of ions in the pore. If such a change occurred at the centre of the pore, the I-V relationship should be symmetrical, as it is for the main channel (Fig. 2a). However, if this change occurred near either end of the pore, an asymmetrical I-V relationship  $^{16,17}$  would be expected. We have found that I-Vrelationships of mini channels are frequently asymmetrical, as illustrated in Fig. 2. Curve a shows the I-V relationship for a single typical channel recorded for 19 sweeps of the I-V curve. Before turning off, the channel converted to a mini channel and the I-V relationship was recorded for six sweeps of the membrane potential (curve b). Curve c shows 11 sweeps of the I-V curve for a single mini channel which did not change state during its lifetime. The broken line is drawn as a reference to demonstrate the asymmetry of the mini channels compared with the symmetry and linearity of the I-V curve of typical channels (curve a).

Although the spontaneous changes between conductance states are infrequent, the occurrence of mini channels which do not change states is rather frequent. This remains the case after extensive purification 6,18-20 and de novo synthesis 20,21 of gramicidin A. Figure 3 shows the relative frequency of minis in the conductance transition histogram. Of the 354 channels that occurred during the experiment, 199 (56%) were mini channels. In other experiments the fraction of minis ranged from 22% to



**Fig. 2** Single-channel I-V relationships for a typical channel, (a, 45.3 pS) and a mini channel into which it converted (c). Also shown is a mini channel which did not change states during its lifetime (b). Purified gramicidin A was added to a glyceryl-1-monoolein/squalene  $(50 \text{ mg ml}^{-1})$  bilayer and a triangular voltage wave (0.5 Hz) applied. Current and voltage were filtered at 100 Hz and sampled simultaneously  $300 \text{ times per s. Baseline-cycle currents were subtracted digitally from currents in cycles where a single channel was conducting and the result plotted against the corresponding measured voltage. The broken reference lines have slopes of <math>22.7 \text{ pS}(c)$  and 3.8 pS(b). 1 M KCl,  $p \text{H} \sim 5.6$ ,  $23 \pm 1 ^{\circ}\text{C}$ .

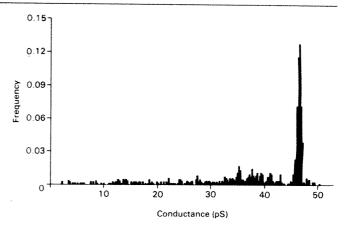


Fig. 3 Histogram of single-channel conductances using HPLC-purified gramicidin A. The sharp peak has a conductance of  $46.0\pm0.3$  (s.d.) pS and contains 44% of the transitions. The remainder are minis which we define as those with a conductance more than two standard deviations below the mean of the main gaussian peak. Glyceryl-1-monoolein (50 mg)/hexadecane (1 ml, Aldrich Gold Label), 1 M KCl,  $pH \sim 5.6$ , 0.1 V,  $23\pm1$  °C.

50%, varying within the expectations based on Poisson statistics. The conductance of minis has a broad distribution over 0-45 pS without any sharply defined features. This implies a profusion of different states whose probabilities of occurrence are roughly the same. The low frequency of spontaneous changes between conductance states ( $<0.0013 \, s^{-1}$ ) indicates that the different states correspond to stable conformations that are separated by large free-energy barriers. A rudimentary calculation, based on absolute rate theory, gives a barrier height of  $\ge 21 \, \text{kcal mol}^{-1}$ .

We found that the rate of channel opening for minis was directly proportional to the rate of channel openings for typical channels over a 1,000-fold range. This indicates that the turn-on frequencies for mini channels and typical channels depend in the same way on membrane gramicidin concentration. As channel turn-on frequency is proportional to the square of gramicidin concentration<sup>16,22,23</sup>, both mini and typical channels must result from the association of two monomers. Thus minis cannot be membrane-spanning monomers, laterally associated trimers or tetramers, or trimers or tetramers in series<sup>6</sup>.

We also found that the mean lifetime of minis does not differ from that of typical channels in either GMO-squalene (40-60 s) or GMO-hexadecane (2-3 s) bilayers. The similar lifetimes of minis and typical channels indicate that (1) minis are not normal channels in thicker patches of membrane<sup>6</sup> and (2) the mini channel structure could not differ greatly from the  $\beta$ -helical<sup>4,10</sup> head-to-head<sup>24,25</sup> dimer structures of typical channels. Were minis of a radically different conformation<sup>26,27</sup> (such as a parallel helix, as proposed by Veatch<sup>26</sup>), their lifetime should also be different from that of the typical channels because a different number of hydrogen bonds must be broken to dissociate such a dimer. Similar arguments rule out other variations of the helical pitch<sup>4</sup> of dimers at the head-to-head junction which would require a different activation energy for dimer dissociation.

Three classes of mechanism for the formation of mini channels would seem consistent with our observations. First, minis could result from the influence of an external factor on the channel, for example a dipolar or charged moiety<sup>16,17</sup> binding tightly to the external surface of one end of the pore and thereby reducing its ion conductance. Second, minis could result from defects in the  $\beta$ -helical structure of the peptide backbone away from the head-to-head junction, as suggested by structural studies<sup>11</sup> indicating that the presence of an ion in the channel may alter the pitch of the backbone helix. Finally, as proposed by Urry et al.<sup>28</sup>, changes in the positions of the side chains, particularly the large tryptophan and leucine R groups, could decrease channel conductance asymmetrically. These side-chain

effects could be mediated by changes in the equilibrium positions of the backbone carbonyls, by changes in the local electric field or the electron-donating capacity of carbonyls, or by changes in the libration energy 10,28 of the protein backbone. If conductance states are determined by side-chain positions, then it is reasonable to speculate that dipolar or reactive residues could couple these states to the electrical potential and/or the presence of agonist molecule, resulting in electrically or chemically gateable channels.

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#### Lateral diffusion of lipids in sarcoplasmic reticulum membranes is area limited

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Scandella et al. used the technique of electron spin resonance to investigate lateral lipid diffusion in the membrane of sarcoplasmic reticulum from skeletal muscle. Their results suggested that lipids are free to diffuse within the plane of the membrane at rates comparable with those in isolated phospholipid bilayer systems of similar composition<sup>2-4</sup>. However, their study<sup>1</sup> was carried out at temperatures between 50 and 70 °C. I have therefore now carried out similar studies, but at physiological temperatures and using fatty acid analogue spin labels. My results show absence of spin-exchange interaction at spin label concentrations below 2-4 mol %. This suggests the existence of diffusion barriers in the plane of the membrane which separate the lipids into clusters containing, on average, about 50 phospholipid molecules.

Table 1 Size of lipid bila	yer clusters an	ers and diffusion coefficients		
<i>T</i> (°C)	n	$D (\times 10^8 \mathrm{cm}^2 \mathrm{s}^{-1})$		
SR membranes				
20	36	1.5		
30	44	1.7		
40	50	2.2		
Sonicated membranes				
20	- Manual Control	1.6		
SR lipids				
20	numerous.	1.7		
40	**********	2.3		

n, Number of phospholipid molecules per bilayer cluster calculated from the concentrations at the break points in Fig. 1b. SR, sarcoplasmic reticulum.

Electron spin resonance (ESR) measurements were carried out between 20 and 40 °C using a stearic acid analogue label, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3oxazolidinoxyl ('5NS'; Syva). This type of spin label has been useful in estimating lateral diffusion rates in Escherichia coli membranes<sup>5</sup>. Its advantage over the phosphatidylcholine analogue used by Scandella et al.1 lies in the relative ease of its introduction into the membrane system, thus avoiding the controllable process of fusion between phosphatidylcholine vesicles and the membrane, which is likely to cause a major structural perturbation in the membrane region where it occurs. The obvious disadvantage of the stearic acid label lies in its structural difference from endogenous lipids.

The method is based on the notion that the collision of spin labels introduced into the fluid lipid phase of the membrane is controlled by diffusion. Since colliding free nitroxide radicals will undergo spin-exchange interaction causing broadening of the ESR lines<sup>2,3,6</sup>, the collision frequency can be monitored by linewidth measurement. Although quantitative evaluation in terms of diffusion coefficients must rely on certain specific assumptions about the diffusion model<sup>4.6</sup>, the observation of concentration-dependent line broadening unambiguously indicates the occurrence of label diffusion.

Figure 1 shows that, within the temperature range used, no concentration-dependent line broadening of the ESR spectrum was observed below certain critical spin label concentrations, indicating that no collisions take place up to these concentrations. Above these concentrations, the linewidth increased, as expected for diffusion-controlled spin label collisions. The same behaviour was found with a different stearic acid analogue label, 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3oxazolidinoxyl (12NS; Syva), while the methyl ester of 12NS showed the same linearly ascending stope at even the lowest concentrations (0.5 mol %).

By adopting the diffusion model and numerical factors described in detail by Sackmann et al.5, the diffusion constants calculated from the slopes above the critical concentrations (Table 1) correspond satisfactorily to the values determined previously for phosphatidylcholine spin labels<sup>1</sup>. This consistency strengthens the reliability of results obtained by stearic acid spin labels. Parallel experiments with extracted membrane phospholipids in aqueous dispersion have verified the fact that at these low concentration increments line broadening could indeed be measured. The slopes observed with the isolated lipid samples were only slightly higher than with the membrane system, confirming that, once diffusion control of collisions prevails, the diffusion rates are very similar in the two systems.

Quantitative evaluation of the hyperfine peaks in terms of the order parameter  $S_3$  and the isotropic coupling constant  $a'_N$  (refs 7, 8) have shown constant values of 0.68 and 14.6 respectively, up to label concentrations of 10 mol %. This eliminates the possibility that the observed absence of collisions is due to binding of the first added spin labels by protein. Therefore, to interpret these effects, I postulate the existence of diffusion barriers in the plane of the membrane separating individual clusters of lipids. Table 1 lists the size of these clusters estimated

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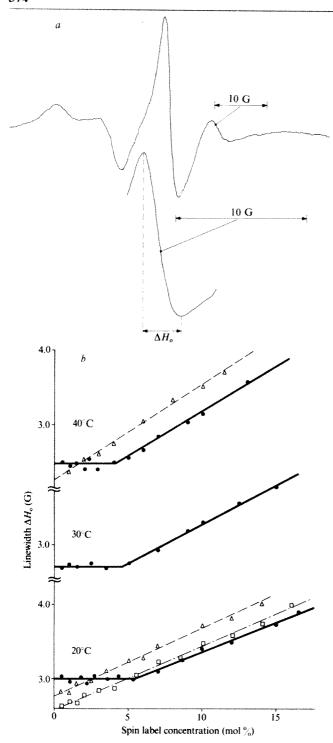


Fig. 1 a, Typical ESR spectrum from stearic acid analogue 5NS in sarcoplasmic reticulum membranes at label concentrations of 1 mol %, relative to total phospholipid (taken as molecular weight 750). Spectrometer settings were as follows. Field set 3,359 G; microwave power 10 mW; receiver gain  $5 \times 10^3$ ; modulation amplitude 0.5 G; recorder time constant 0.5 s; scan time 4 min. The central line was recorded in a scan range of 40 G to facilitate precise linewidth measurement. A Varian E 104-A X-band spectrometer with variable temperature control unit was used. Sarcoplasmic reticulum membranes were prepared according to previously documented methods 10,11 and studied within 48 h of preparation. Spin labels were introduced by gently shaking membrane suspensions, typically containing ~20 mg ml pholipids, under N<sub>2</sub> atmosphere in a glass tube containing the appropriate amounts of dry label deposited on the wall. b, Linewidths of the central ESR line of 5NS in sarcoplasmic reticulum membranes (●), isolated membrane phospholipid suspensions (△) and a sample of sarcoplasmic reticulum that had undergone 1 min ultrasonication ( ) using the 1-cm probe tip of a Braun Labsonic sonicator.

from the critical onset concentrations for line broadening. For simplicity, this calculation involves the assumption that the clusters are phospholipid bilayers, that is, contain twice the number of molecules as deduced from the critical concentrations.

At physiological temperatures, the clusters would contain, on average, 50 phospholipid molecules, which is about half the of phospholipids per molecule of (Ca<sup>2+</sup>+ number Mg<sup>2+</sup>)ATPase, the major integral protein constituent of the membrane. It could be speculated that this reflects the fraction of phospholipids that are not involved in forming an 'annulus' around the ATPase which, by definition, would diffuse at a considerably slower rate.

The above estimations imply uniform contributions to the measured linewidths by all spin labels. This is supported, at concentrations <5 mol %, by the absence of any free-label signal, indicating that all spin labels are incorporated into the membranes. Even above this concentration, the area under the subsequently gradually occurring free-label signal is only a minor fraction of the total and hence safely negligible for the present considerations. On the other hand, it cannot be excluded that a small number of spin label molecules are immobilized by binding to protein below the detection level. However, this also is negligible in relation to the concentrations relevant here.

Although it is premature to speculate on the detailed nature of the diffusion barriers, the finding that neither lipids alone nor ultrasonically perturbed membranes show these effects suggests that protein-protein interaction is probably involved. The observation that only the free stearic acid analogues and not the methyl ester are sensitive to this effect furthermore suggests that ionic interactions might be relevant in defining the limits of lipid diffusion. Note that the present results allow direct conclusions only to negatively charged lipids. Future experiments must show whether this also holds true for positive or zwitterionic lipids.

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#### Variant insertion element IS1 generates 8-base pair duplications of the target sequence

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Transposition in both eukaryotes and prokaryotes is characterized by the generation of short, direct duplications of the target DNA sequence at the integration site of the transposable element<sup>1</sup>. For example, the bacterial insertion sequence IS1, or

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the IS1-flanked transposon Tn9, generates 9-base pair (bp) repeats during either transposition or cointegration  $^{2-8}$ . All the transposable elements so far sequenced are characterized by the presence at their ends, of a perfect, or near-perfect, inverted repeat sequence, which is ~30 bp long and is thought to be important for recognition by enzymes during transposition. While studying IS1-mediated cointegration  $^{9-12}$ , we found an IS1 carrying a single base pair change in the terminal inverted repeat sequence. We now report that it generates 8-bp duplications of the target sequence, a finding which confirms the importance of the inverted repeat for transposition and which, because one end only of the IS1 is changed, has implications for the mechanism of transposition by IS1.

We have characterized four pBR325 derivatives carrying IS I, all of which resulted from segregation of cointegrates between pBR325 and plasmid P1, which contains an IS I element 11-13 (Fig. 1). The sites of insertion and the orientation of the IS I in these different derivatives have been described previously 11,12, and the sequencing strategy used is shown in Fig. 2.

Figure 3 shows the results of the DNA sequence analysis around the junctions of IS1 and pBR325. In each plasmid, the IS1 had integrated at a different position within a 300-bp region of pBR325 between coordinates 4,134 and 4,411 (ref. 14). Two of the plasmids (pSHI211 and pSHI212) carried the 9-bp duplications of target sequence previously reported for IS1<sup>2-8</sup> but the other two (pSHI213 and pSHI214) had only 8-bp

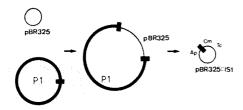
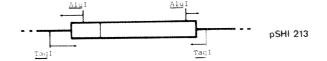


Fig. 1 The formation of transpositional cointegrates. Phage P1 was induced in P1-lysogenic Rec<sup>+</sup> bacteria containing pBR325, which codes for resistance to ampicillin (Ap), chloramphenicol (Cm) and tetracycline (Tc) <sup>14,31</sup>, and cointegrates were isolated as specialized P1 transducing phages. Their structure is shown in the centre of the figure: the P1 genome (thick line) has integrated into the pBR325 (thin line) and is flanked by direct repeats of the IS1 element resident on the P1 genome (bar). Subsequent resolution of these cointegrates in Rec<sup>+</sup> cells yields a pBR325 derivative carrying a single copy of IS1<sup>11,12</sup>.

duplications. The IS element of the latter two has a G to T transversion at position 757, 12 bp from the end of the element, and no other alteration within 50 bp of the end when compared with the canonical sequence<sup>3.15,16</sup>. The same alteration has also been found in one of the flanking IS 1s of  $Tn9^8$ . There seems to be a good correlation, in our experimental conditions, between the alteration in the inverted repeat of IS 1 and the ability of the element to generate 8-bp repeats.

The transposition characteristics of the variant IS I seem very similar to those of IS I itself. For example, in both cases the preferred sites for IS I integration are A+T rich<sup>5-8,17-19</sup> and both elements show a strong preference for inserting in sites that have a G-C base pair at each end of the target sequence to be duplicated<sup>8</sup>. The variant IS I stimulates the formation of deletions, as does the wild type, and transposes with a slightly higher frequency I 1,12.

IS1-mediated transpositional cointegrations have been observed in various experimental systems  $^{7,9,11,12,20}$ . As only one of the two parental plasmids originally contained an IS1 and the resulting cointegrates carry two IS1s at the junction of the replicons (Fig. 1), one can assume that the IS1 duplicates during



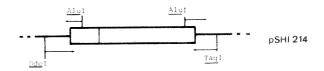


Fig. 2 Sequencing strategy for the variant IS1 ends. IS1 is drawn as a box and the flanking DNA sequences as a horizontal line; the vertical line within the box shows the orientation of IS1 and indicates the position of the single Ps1 site. Plasmids pSHI213 and pSHI214 had IS1 integrated into pBR325 at locations 4,404–4,411 and 4,134–4,141 respectively, and were sequenced as indicated by arrows. The other two plasmids, pSHI211 and pSHI212, had IS1 integrated at positions 4,202–4,210 and 4,248–4,256 respectively, and the ends were sequenced using the AluI sites of IS1. DNA sequencing was done using the method of Maxam and Gilbert<sup>32</sup>.

the process. If this is the case, the IS1 elements carried by the four pBR325 derivatives that we have analysed ought to have the same DNA sequence as the IS1 of P1. However, we find that two of the four carry an alteration. The possibility that the P1 parental phage carried heterogeneous IS1s is less likely because the cointegrates were isolated from fresh lysogens derived from a single plaque. Alternatively, a chromosomal IS1 may first have transposed into pBR325 and the cointegrates then have arisen by reciprocal recombination between the IS1 acquired by pBR325 and the IS1 of P1; such a pathway has recently been demonstrated in Rec<sup>+</sup> cells<sup>12</sup>. The most likely explanation is that two of the four cointegrates studied were formed by this pathway while the other two were formed by the IS1 duplication process. The same G to A transversion has been found in the flanking IS1 of Tn9 carried on  $\lambda cam1^8$  and this Tn9 originally derived from P1Cm0, which has Tn9 inserted within the IS1 of P1 (refs 13, 21-23). Therefore, the variant IS1 probably comes from the P1 genome.

The recently proposed models for transposition fall into two groups differing in the mechanism of transposition initiation. In group I models, only one end of the IS element is ligated to the target sequence at the initiation step $^{24-26}$ , whereas group II models predict that both ends of the IS element are ligated $^{27.28}$ . A cointegrate is a necessary intermediate in group II models whereas it is a by-product in group I models. Evidence supporting transposition of IS 1 by a group I mechanism comes from the study of IS 1-mediated cointegrates. Their formation seems to be less frequent than transposition, but once formed they are

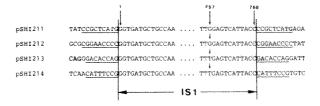


Fig. 3 DNA sequence of the ends of IS1 and flanking sequences. The ends of the IS1 are delineated by vertical lines. Duplicated plasmid sequences are underlined. Arrows indicate the position of the variation in the terminal inverted repeat of IS1 carried by pSHI213 and pSHI214. IS1 coordinates are from ref. 15.

stable, even in recombination-proficient cells<sup>7,10-12</sup>, which argues that they are not intermediates in transposition. This contrasts with some other transposons, such as Tn3, where the cointegrates are unstable 28-30; such transposons probably use a group II mechanism. Our observation that an alteration in one end only of IS1 changes its transposition characteristics also favours a group I mechanism. In this respect, it is interesting that the Tn9 with the same base alteration has been shown to generate 9-bp repeats<sup>4,8</sup>. It is unlikely that the repeat generated depends on whether the process is transposition or cointe-

gration. The end of the IS element chosen to initiate transposition may be dictated not only by the sequence of the IS element itself, but also by sequences in the donor DNA molecule, perhaps the recognition sequences in the donor that were used when it previously acquired the IS element.

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#### Promoters in the E. coli replication origin

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Chromosomal replication in Escherichia coli initiates at a particular sequence in the parental DNA called the origin of replication (oriC) and proceeds bidirectionally. Initiation requires the interaction of various factors at a given time within chromosomes have been constructed<sup>4-6</sup> and partially sequenced<sup>7,8</sup> whose replication sequenced<sup>7,8</sup> whose replication proved also to be dependent on a rifampicin-sensitive host function in addition to the products of several initiation genes<sup>6,9</sup>. Using one of these mini-chromosomes, pCM959<sup>6,8</sup>, we have searched for promoters in and close to oriC to elucidate the role of RNA polymerase in the initiation of replication. We have found, by in vitro transcription experiments and RNA sequencing, that the DNA segment required for the start of bidirectional replication in E. coli contains two promoters arranged back-to-back. The location and arrangement of these promoters suggest an involvement in the initiation of replication.

Several complementary techniques were used to identify promoters likely to be involved in the initiation of replication. First we surveyed for promoters by mapping RNA polymerase binding sites present on pCM959 using electron microscopy. Three RNA polymerase binding sites, at nucleotide positions  $267 \pm 61$ ,  $791 \pm 55$  and  $3,991 \pm 68$  of pCM959, mapped close to or within oriC; details will be published elsewhere

We used an in vitro transcription assay to determine the direction of transcription, the precise location of the transcription starts and the relative efficiencies of these promoters, which presumably have different functions. Various restriction fragments from pCM959 encompassing the three RNA polymerase binding sites of interest were used as templates for

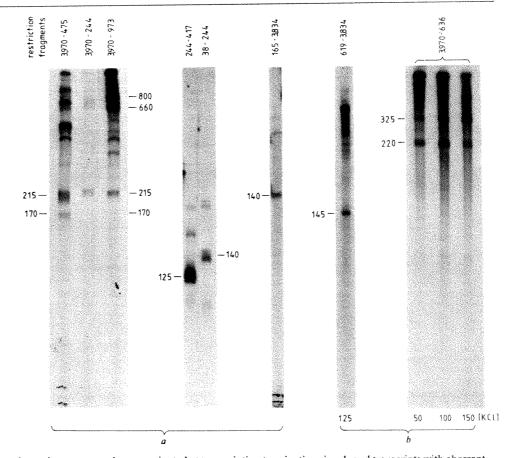
purified RNA polymerase. The products of such reactions are

Figure 2 shows the alignment of transcripts that end at the termini of these restriction fragments (run-off transcripts). For example, using the HaeIII fragment (position 3,970 to 636) as template, transcripts are obtained that are 220 and 325 nucleotides long (Figs 1b, 2). When this fragment is cleaved with Hhal (position 475) or HindIII (position 244), the 220 (215)-nucleotide transcript is still present, but instead of the 325-nucleotide transcript, a shorter length of 170 nucleotides is found, or, in the case of HindIII cleavage, the transcript is missing entirely (Figs 1a, 2). A 125-nucleotide transcript, however, is obtained if the HindIII-XhoI fragment (position 244 to 417) is assayed. The results obtained with the different fragments (Fig. 2) suggest that the RNA polymerase binding site observed at position  $267 \pm 61$ is in fact due to two promoters situated back-to-back, which we designate Pori-1 and Pori-r. According to this analysis the RNA origins should be located at position 170±7 for Pori-1 with counterclockwise transcription, at position 310 ± 10 for Pori-r with clockwise transcription, at position  $765 \pm 7$  for the binding site at position  $791 \pm 55$  with counterclockwise transcription, and at position  $3,980 \pm 10$  for the binding site at  $3,991 \pm 68$  with counterclockwise transcription.

To determine precisely the transcriptional starts, and to confirm the alignment, various 5'-end-32PO4-labelled transcripts obtained from restriction fragments encompassing both origin promoters were subjected to RNA sequence analysis 11-14. The RNA sequences were then compared with the DNA sequence. Transcripts originating from Pori-1 started mainly with C at position 166 (Figs 3, 4). Transcripts originating from Pori-r started mainly with A at position 323 (Figs 3, 4); some transcripts from Pori-r, however, started with G at position 313 (complete sequencing data will be published elsewhere).

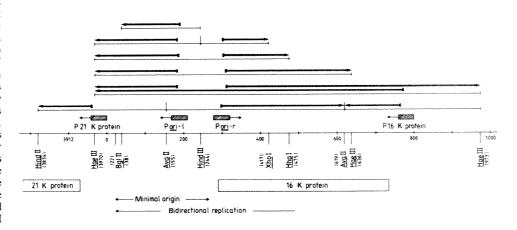
The directions of transcription determined for the promoters outside oriC agree with those inferred from the pCM959 nucleotide sequence (Fig. 3). These two promoters are followed by a Shine-Dalgarno sequence<sup>15</sup> within the postulated distance<sup>16</sup> of an open reading frame for proteins with molecular weights (MWs) of 16,000 (ref. 7) and 56,000, respectively (Fig. 3). The function of the 16,000-MW protein is unknown but it does not seem to have a major effect on minichromosome

ctrophoretic analysis of synthesized by purified E. polymerase holoenzyme rious restriction fragments passing oriC. The transcripxperiments were carried out in nal volume of 200 µl with 0.05μg purified DNA fragment and -15 µg RNA polymerase (85 mol RNA polymerase per mol DNA fragment<sup>28</sup>) in 40 mM Tris-HCl pH 8.0, 10 mM MgCl<sub>2</sub>, 4 mM dithiothreitol, 400  $\mu$ M ATP and pH 8.0. GTP, 100  $\mu$ M CTP and UTP+50-100  $\mu$ Ci[ $\alpha$ - $^{32}$ P]UTP with a, 50 mM KCl and 100  $\mu$ g ml<sup>-1</sup> heparin<sup>29,30</sup> or b, 50, 100 or 150 mM KCl (ref. 28). Reaction mixtures were preincubated for 10 min at 37 °C in the absence of a, UTP, CTP and heparin, or b, MgCL<sub>2</sub> (ref. 28). The complete reaction mixture was incubated for 20 min at 37 °C and the reaction stopped by addition of phenol. Samples were precipitated with ethanol, dissolved in 50% formamide, treated for 2 min at 100 °C, and subjected to electrophoresis in 8% (a) or 10% (b) polyacrylamide gels containing urea<sup>31</sup>. Numbers above the tracks specify the coordinates of the restriction fragments. Numbers alongside major transcripts represent their size in bases. 32Plabelled DNA fragments served as



length standards. Transcripts having no size assignments are those terminated at transcription termination signals and transcripts with aberrant electrophoretic behaviour, as evidenced by the nucleotide sequence of their 5'-terminal portions.

Fig. 2 Alignment of transcripts obtained from restriction fragments containing oriC and/or adjacent DNA segments. According to the length determination specified in Fig. 1, major transcripts (thick lines) were arranged by aligning their 3 ends with the ends of restriction fragments (thin lines). The positions of the promoters are indicated by cross-hatched boxes. Arrows indicate transcription directions. Numbers next to restriction sites within the physical map specify their coordinates. The open boxes beneath the physical map specify the coding regions of proteins. Both the limits of the minimal origin<sup>23</sup> and the region required for bidirectional replication<sup>24</sup> are shown. The *HaeIII* (636) site is only present in a derivative of pCM959.

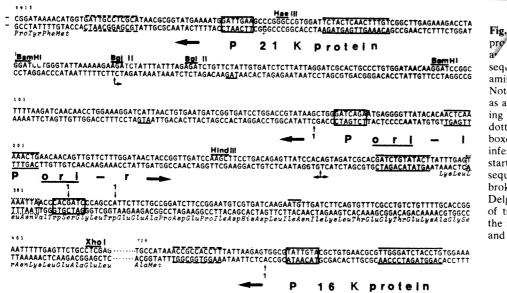


replication<sup>17</sup>, even though its coding region overlaps partially with that of transcripts from Pori-r. The 56,000-MW protein is a fusion product of a 21,000- and a 35,000-MW partial peptide as its coding region traverses the point of circularization of pCM959 (data not shown). The 21,000-MW partial peptide may be the beginning of a 70,000-MW protein designated gid, which was mapped in this region<sup>17</sup>. Both a 16,000- and a 70,000-MW protein have been seen after infection of minicells carrying the  $\lambda$  repressor-overproducing plasmid pGY101<sup>18</sup> with  $\lambda$  vectors into which different minichromosomes were inserted (unpublished results), and in cells carrying minichromosomes<sup>19</sup>. Both these promoters were also demonstrated in vivo<sup>19</sup>.

Although these observations suggest a linkage between transcription and translation for the two promoters outside oriC, a

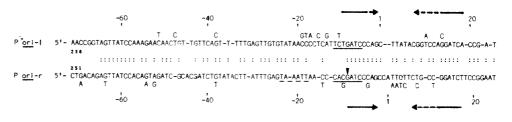
coupling of these two processes for the two oriC promoters seems unlikely. Nonsense codons are present in all reading frames, and the longest possible peptides which can be deduced from the nucleotide sequence downstream of Pori-1 and Pori-r are 27 amino acids long (130-50) and 55 amino acids long (351-525), respectively (Fig. 3). Such peptides have so far not been identified. The origin promoters seem to be as active in vitro as the 16,000-MW protein promoter and far more active than the 21,000-MW partial protein promoter, which expresses transcripts that are barely above background. Therefore we suggest that these promoters are also used in vivo, but for a purpose other than protein biosynthesis.

When the nucleotide sequences of the two origin promoters are compared, we observe extended sequence homologies



pro sequ amino Note that n. as a transcript a ing Pribnow box dotted line. † Transc. boxes indicate Pribnow . inferred from the transcra, starts<sup>20</sup>; lines above and below t<sub>1</sub>, sequences represent -35 regions<sup>20</sup>; broken lines indicate Shine-Delgarno sequences<sup>15</sup>.  $\rightarrow$ , Direction of transcription;  $\leftrightarrow \leftarrow \uparrow$ , limits of the minimal origin segment<sup>23</sup>. Start and stop codons downstream of Poril and Pori-r are underlined.

Fig. 4 Comparison of origin promoters of E. coli and S. typhimurium. The sequences of the noncoding strands starting at base 238 and 251, respectively, are compared. Numbers above below the sequences refer to nucleotide positions relative to the transcriptional start. The 'secondary' Pribnow box occurring in Pori-r is marked by dashes; ▼ represents its



corresponding 5' start nucleotide. The corresponding S. typhimurium sequence 21 is shown above or below the E. coli sequence only where there are sequence differences. : indicate identical bases in the two E. coli promoter sequences. Note that most changes within the promoter sequences are found in regions which are also more variable in other promoters. The changes are located between the Pribnow boxes (underlined regions) and around base -20 (ref. 20). Other changes occur within the loop of a putative operator structure indicated by inverted arrows.

around position -50 to -70 and close to the transcription starts (Fig. 4). The region preceding the actual promoter sequence is known to be important for positive control in the lac, gal and other systems<sup>20</sup>. This sequence homology is also evident when these regions are compared with the corresponding segment in the Salmonella typhimurium origin (ref. 21; Fig. 4) and with the other origins of Gram-negative bacteria so far sequenced, for example Klebsiella pneumoniae, Enterobacter aerogenes and Erwinia carotovora<sup>22</sup>. Thus an attractive hypothesis is that such RNA synthesis occurs in all these Gram-negative bacteria and that it is subject to positive control in all cases. The second conserved sequence homologies are close to the transcription starts. These regions of homology can form imperfect palindromes and may therefore represent sites of interactions, occurring on both promoters, with a hypothetical repressor.

The minimal DNA segment required for origin function extends from position 22 to position 267 (ref. 23; Fig. 3) whereas oriC, the origin required for bidirectional replication, has been shown to extend beyond position 417 (ref. 24). Pori-1 is thus completely contained in the DNA segment that is sufficient for leftward unidirectional replication. Pori-r is located in that part of oriC which is, in addition, required for bidirectional replication. This arrangement suggests that the origin promoters are responsible for the synthesis of RNA primers required for the inception of the leading DNA strands. However, we cannot rule out the possibility that Pori-1 and Pori-r promote, together or exclusively, transcriptional activation<sup>25</sup> or that one or both transcripts have a regulatory role similar to that of the short RNA molecules found in some plasmid systems<sup>26,2</sup>

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# Molecular dynamics of hydrogen bonds in bovine pancreatic trypsin inhibitor protein

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Molecular dynamics simulations starting from the X-ray structure<sup>1</sup> of bovine pancreatic trypsin inhibitor protein (BPTI) reveal that the different hydrogen bonds observed in the X-ray coordinates have different stabilities as indicated by the mean lengths and length fluctuations. The most stable hydrogen bonds involve the hydrogen atoms observed to exchange most slowly with solvent in NMR experiments<sup>2</sup>, and to have the shortest lengths in the X-ray structure. This agreement between calculation and experiment suggests that molecular dynamics simulations can complement X-ray studies by providing reliable information about the rates and pathways of conformational changes about the mean positions observed in protein crystals.

The results of dynamics simulations depend on the molecular potential energy from which the forces acting on the atoms are calculated. As all force calculations on proteins<sup>3-5</sup> use an approximate empirical potential energy function and omit the thousands of surrounding solvent molecules, it is essential to check the results quantitatively before using such theoretical

methods to calculate quantities that cannot be measured. Two observable properties of globular proteins seem particularly well suited to such a test: (1) the amplitudes of atomic vibration available from the highest-resolution X-ray studies can be compared with the amplitudes computed from a dynamics run; and (2) the rates at which hydrogen atoms in the protein exchange with deuterium atoms in solution, as measured by <sup>1</sup>H NMR, can be compared with the computed fluctuations in the hydrogen bond lengths. Northrup et al. <sup>6</sup> have shown that the observed and calculated atomic vibration amplitudes yield similar pictures for the atomic mobility in the protein ferrocytochrome c.

Here I analyse the results of molecular dynamics simulations on the protein BPTI in terms of the length fluctuations of hydrogen bonds. BPTI is the obvious choice for this study as the rates of exchange of individual protons are not available for other globular proteins<sup>2</sup>. In the calculation, the equations of motion in the protein force field used previously<sup>5,7</sup> are solved by the Beeman<sup>8</sup> method of numerical integration with a time step of  $2 \times 10^{-15}$  s. Rapid equilibration is achieved by starting from a completely relaxed structure generated by extensive conjugate gradient minimization<sup>5</sup> (up to 3,000 energy evaluations) from the X-ray structure and then gradually increasing kinetic energy by small random impulses to give an equilibrium velocity distribution (gaussian) with no local 'hot spots'.

The mean lengths and length fluctuations of hydrogen bonds calculated from a 56-ps molecular dynamics simulation (see Table 1) are insensitive to the part of the trajectory from which they are calculated (correlation coefficient of mean lengths in MD1 and MD2 is 0.98), indicating that the trajectory is well equilibrated for calculation of these quantities. The different hydrogen bonds in the structure show systematic differences in mean lengths and length fluctuations allowing all 24 hydrogen bonds in Table 1 to be classified as 'stable', if the mean length (the O—H distance) is <1.90 Å and the r.m.s. length fluctuation

X-ray

MD1 (1-24 ps) MD2 (32-56 ps) length exchange rate Secondary structure and Mean hydrogen bond Mean Amp Class (Å)  $(min^{-1})$  $\alpha$ -helix Pro 2, O-Cys 6, H 2.11 0.32 2.23 0.47 U 2.25 0 2.12 Asp 3, O-Leu 7, H 2.17 () 2.15 0.33 U 0.56 B-hairpin Thr 11, O-Gly 36, H 0.11 1.81 0.10 1.83 2.19 Gly 36, O-Ala 16, H U 4.01 0.50 4.10 2.64 1.79 Tyr 35, O-Ile 18, H 0.63 2.38 0.65 U 2.21 Ile 18, O-Tyr 35, H 1.78 0.09 \$ \$ \$ \$ \$ \$ \$ 1.68 0.10 Phe 33, O-Arg 20, H 1.80 0.09 1.80 0.10 2.05 Arg 20, O-Phe 33, H 1.79 0.09 1.80 2.00 0.10 Phe 45, O-Tyr 21, H Tyr 21, O-Phe 45, H 1.81 1.77 1.82 1.79 0.10 0.10 1.71 --6 1.76 0.08 -6 0.09 Gln 31, O-Phe 22, H 0.10 1.82 0.11 1.90 Phe 22, O-Gln 31, H 1.76 0.08 1.77 0.08 1.96 1.85 S 2.02 Leu 29, O-Asn 24, H 1.81 0.10 0.15 1.81 Asn 24, O-Gly 28, H 1.82 0.10 1.82 0.10 2.06 0 Ser 47, O-Cys 51, H Ala 48, O-Met 52, H 2.74 0.602.33 0.45U 2.13 0.21 1.89 1.88 0.18 S U 1.82 --4 Asp 49, O-Arg 53, H 2.73 2.56 0.62 2.12 0 0.64 Asp 50, O-Thr 54, H 3.28 0.57 3.07 0.44 U Cys 51, O-Cys 55, H Met 52, O-Gly 56, H 1.81 0.12 1.82 0.16 S U 1.83 3.35 0.44 3.41 0.321.94

Table 1 Calculated and observed properties of hydrogen bonds

Mean length (A)

The amplitude (amp.) is the r.m.s. deviation of the hydrogen bond length from the mean value for the specified part of the trajectory.  $\log_{10}{}^{1}$ H exchange rates are taken from Table 1 in ref. 2. The rapidly exchanging protons are not seen in the experiment and are given the rate of  $1 \text{ min}^{-1}$ , corresponding to that of an exposed amide group in model peptides<sup>14</sup>. U, unstable; S, stable.

1.82

1.86

1.99

1.80

0.13

0.14

0.22

0.10

S

S

Ŭ

1.84

2.03

2.65

 $0028 \hbox{--} 0836 / 81 / 480379 \hbox{---} 02\$01.00$ 

Side chain/main chain Glu 7, O-Asn 43, HD1 Tyr 23, O-Asn 43, HD2

Asn 24, OD1-Lys 26, H

Asn 43, OD1-Tyr 23, H

1.93

1.83

0.32

0.13

0.20

-- 5

0

log<sub>10</sub> <sup>1</sup>H

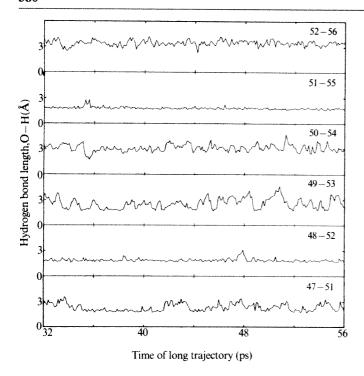


Fig. 1 The time variation of the six O—H distances of the peptide hydrogen bonds observed in the C-terminal  $\alpha$ -helix of BPTI during the period of 32-56 ps of the trajectory. A well-formed hydrogen bond has a length of 1.7-2.2 Å.

(amp.) < 0.22 Å (marked 'S' in Table 1), or 'unstable' otherwise (marked 'U'). Comparison with the experimentally determined X-ray lengths<sup>1</sup> shows that the 'stable' hydrogen bonds are significantly shorter: 15 of the 16 X-ray lengths <2.07 Å correspond to hydrogen bonds defined as stable by the independent criteria given above. Comparison with the <sup>1</sup>H proton NMR exchange rates measured for peptide protons<sup>2</sup> shows that the 'stable' hydrogen bonds have significantly slower proton exchange rates: 14 of the 15 most slowly exchanging protons found experimentally correspond to stable hydrogen bonds (S in Table 1).

This agreement between the classification of the 24 hydrogen bonds on the basis of the dynamics simulation and either the X-ray lengths or the NMR exchange rates is highly (99.99%) significant. If the 24 hydrogen bonds were to be randomly assigned as 'S' or 'U' subject to the requirement that there be exactly 15 'S' assignments, there would be 24!/(15! 9!) distinct arrangements. One of these would match the NMR exchange rates exactly and 9 × 15 would have two mismatches, as is found here. The probability of getting chance agreement as good as that seen here is  $(9 \times 15 + 1)/(24!/(15! 9!)) = 136/1,307,504 =$ 0.0001.

Despite this good agreement, the correlation between the mean lengths (or length fluctuations) and the actual exchange rates of individual hydrogen bonds is poor (for example, both Phe 33, O-Arg 33, H and Thr 11, O-Gly 36, H are correctly classified as stable but have exchange rates that differ by five orders of magnitude). Thus although the calculation can successfully predict which of the hydrogens will exchange slowly, it cannot predict the actual exchange rates. This is to be expected as hydrogen exchange occurs on the time scale of minutes and depends on many factors besides the stability of the hydrogen bond, including local unfolding, solvent penetration and catalysis by neighbouring groups.

Why are certain hydrogen bonds unstable as measured by the three independent criteria of dynamics simulation, X-ray lengths and NMR exchange rate? One explanation is that the

potential energy of certain hydrogen bonds is high (less favourable) due to local conformational strain. Another is that certain hydrogen bonds fluctuate more to increase their conformational entropy and so reduce the free energy of the system. The entropy gained from larger fluctuations depends on the density of packing around the hydrogen bond in question: if there is no space to move into, there is nothing to be gained from large fluctuations. Support for this idea comes from the accessibilities of the bonds (calculated as the sum of the static accessible surface areas of the O and N atoms in the X-ray structure): 5 of the 9 'U' hydrogen bonds in Table 1 are exposed (3, O-7, H =  $8.3 \text{ Å}^2$ ; 36, O-16, H =  $3 \text{ Å}^2$ ; 24, O-28, H =  $0.3 \text{ Å}^2$ ; 49, O-53, H =  $7.2 \text{ Å}^2$ ; and 50, O-54, H =  $37.8 \text{ Å}^2$ ) whereas only 2 of the 15 'S' hydrogen bonds are exposed (48, O-52, H =  $7.7 \text{ Å}^2$ and 24, OD1-26,  $H = 9.8 \text{ Å}^2$ ).

Figure 1 shows the time variation of the six peptide hydrogen bond lengths in the C-terminal  $\alpha$ -helix. The two stable hydrogen bonds (Ala 48, O-Met 52, H and Cys 51, O-Cys 55, H) vibrate mostly about the length value of 1.8 Å, but occasionally show increases in length of up to 3 Å for periods of  $\sim 0.5$  ps; these fluctuations do not involve the correlated breaking of hydrogen bonds adjacent along the  $\alpha$ -helix. The four unstable hydrogen bonds (Ser 47, O-Cys 51, H; Asp 49, O-Arg 53, H; Asp 50, O-Thr 54, H; and Met 52, O-Gly 56, H) show larger length fluctuations, but a good hydrogen bond is sometimes formed for a few picoseconds. The same type of dynamic behaviour is seen for the other hydrogen bonds and for other periods of the trajectory.

Discussion of the broad range of rates with which protons exchange in globular protein has in the past focused on how the particular groups contact the solvent 10-12,13. My study emphasizes another factor showing that the intrinsic stability of different hydrogen bonds differs and that the stable hydrogen bonds exchange more slowly.

This work differs from previous studies of BPTI dynamics<sup>3,4</sup> in two important respects. (1) I allow hydrogen bonds to be formed between all oxygen and hydrogen atoms that are close together in space. As the previous studies used a fixed list of hydrogen bonds taken from the X-ray data<sup>1</sup>, questions concerning hydrogen bond fluctuations could not be answered. (2) I use a different potential energy function, initially calibrated on the observed unit cell parameters of hydrocarbon, amide and amino acid crystals and then tested for its ability to give an equilibrium conformation close to the observed X-ray structure<sup>5</sup>. The present simulations do, however, have a serious shortcoming in the omission of the thousands of water molecules surrounding the protein molecule: 2,700 water molecules would have to be included to give a 0.02 M solution (very concentrated by experimental standards), increasing the computer time for one time step by a factor of ~10. This more realistic calculation is now in progress.

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# **BOOK REVIEWS**

# Environmental impact re-assessment?

#### **David Pearce**

THE current fashion for environmental impact assessment (EIA) is partly explained by the continuing force of the environmental protection movement in Western countries. That movement is now under severe pressure from economic recession, and there are signs that impact assessments themselves will play a decreasing role in planning and development. Certainly, this is the message that emerges from the USA, where the emphasis is switching back to the costs of environmental protection. This reverse swing, no sign of which is even hinted at in the two volumes under review, is both unfortunate and misguided. But the environmental movement itself bears some of the responsibility. In their indecent rush to find an approach (some might say any approach) to the integration of environmental factors with the social and economic dimensions of development. environmentalists have produced a jumble of methodologies all too few of which have any rational grounding.

In essence, an EIA should identify all environmental and social impacts from a development. It may then leave them qualitatively recorded, or go further and measure them in physical terms or as "scores" reflecting a judgement of whether that impact is good, bad, moderate or serious. Neither Project Appraisal and Policy Review, which is a collection of essays on the state of play in EIA, nor Environmental Impact Assessment, the proceedings of the 1979 meeting of the UN Economic Commission for Europe (ECE), will aid anyone unfamiliar with the methodologies and techniques used for EIA. The ECE volume does, however, highlight the prevailing chaos whereby almost anything that quantifies, indicates or even mentions "impacts" is classified as an EIA. The conference lamented this state of affairs and one of its recommendations is for systematization. But there is a surprising lack of self-criticism in both books. Some of the scoring methods, for example, actually offend logic by placing cardinal numbers on ordinal rankings. Moreover, the call for systematization is negated by fairly regular references to the "flexibility" of EIA, as if this is a virtue rather than a euphemism for arbitrary procedures.

Both volumes would have greater claim to authority had they included contributions from economists. One example will suffice. In the USA the National Environmental Protection Act mandated EIA for public sector investments. A fairly Project Appraisal and Policy Review. Edited by Timothy O'Riordan and W. R. Derrick Sewell. Pp. 304. ISBN 0-471-27853-X. (Wiley: 1981.) \$36, £13. Environmental Impact Assessment. Proceedings of a Seminar of the United Nations Economic Commission for Europe. Pp.368. ISBN 0-08-024445-9. (Pergamon: 1981.) \$55, £23.

traditional cost-benefit analysis was to be included. Without it, one may observe, it is not clear how anyone is to decide whether the investment is worth while at all. Yet O'Riordan and Sewell speak of the overnarrow concern in cost-benefit analysis with "economic efficiency" while their coauthors speak of the deficiencies of planning procedures that cannot debate national "need", which is what economic efficiency is all about.

A further flaw is that the value system underlying EIA is not investigated in either book. Cost-benefit analysis attempts to reflect an aggregate of individuals' preferences. In EIA, though it is hardly acknowledged, it is the planners' preferences that count. Possibly the unease which a democrat should feel in this respect explains the emphasis given by some of the authors in the O'Riordan and Sewell volume to the need to link EIA to "public involvement", although one cannot be quite sure who the "public" are. An EIA that finally emerges as a matrix of impacts does at least provide information for local and central government, as the authors correctly emphasize. But that same matrix - consisting of incommensurable impacts, subjective assessments by planners and consultants, objective physical measures

which may or may not be disputed by experts — cannot then answer questions of social worth unless reduced to commensurable terms with the demands the development makes on resources, i.e. its money cost. Moreover, EIA is of limited value in assisting choice between alternatives unless the impacts in the matrix for one assessment are systematically greater or less than the impacts in the assessment of the alternative.

The most provocative essay in the O'Riordan and Sewell volume, by Nick Abel and Michael Stocking, questions whether EIA has any relevance to less developed countries. Their argument is basically that EIA enshrines Western values, thus providing inappropriate evaluations of development projects, for example by implicitly equating low economic output per capita with "backwardness". Their strictures are familiar but worthy of careful examination. Anyone who has worked in low per capita income countries will doubt if Westernized assessment procedures are quite so irrelevant, since the general aim of income expansion is wholly warranted. But there is an unquestionable need to place EIA and other appraisal techniques in the political context of the country in question.

To anyone looking for an "update" on the state of play in EIA these are useful volumes, *Project Appraisal and Policy Review* especially so. Sadly, however, neither book has come to terms with the underlying questions which any project appraisal needs to ask.

David Pearce is Professor of Political Economy at the University of Aberdeen.

#### Lives and work of the nervous doctors

W.F. Bynum

The Doctrine of the Nerves: Chapters in the History of Neurology. By John D. Spillane. Pp. 467. ISBN 0-19-261135-6. (Oxford University Press: 1981.) £25, \$50.

THE title of Dr Spillane's volume comes from Thomas Willis's (1621–1675) original definition of "neurologie" as the branch of medicine dealing with "the doctrine of the nerves". The word, of course, has since changed in meaning, and long before Willis the central nervous system and its associated nerves had been recognized as a

crucial custodian of our uniqueness: the recepticle of our sensations, originator of our movements, storehouse of our memories, the seat of our souls. Consequently, the study of the structure, functions and derangements of the nervous system has a rich history, as this elegantly written and beautifully illustrated book attests.

Unlike Caesar's Gaul, Dr Spillane's book is divided into four parts. He describes the classical foundations (Galen, Vesalius, Willis), the eighteenth-century obsession with the nervous system as the origin of most disease, the nineteenth-century establishment of experimental neuroscience and, finally, the flowering of clinical neurology in the late nineteenth century. The approach is primarily biographical and expository, which permits selectivity and places the value of the work on its individual sections rather than on any unifying synthesis. As the sub-title suggests, the book is a series of chapters rather than an integrated history.

This approach has its limitations and its strengths. Among the latter is the way it permits Spillane to bring his own neurological knowledge to bear on the ideas of his intellectual forebears. This is particularly true for the more recent neurologists such as G.B.A. Duchenne, W.R. Gowers and Silas Weir Mitchell, men whose names are invoked more often than their works are read. Spillane has also rehabilitated several men whose contributions are often forgotten: John Cooke (1756-1838) and John Russell Reynolds (1828-1896) fall into this category. Nearly 200 illustrations are neatly integrated into the narrative, and production standards are high.

On the other hand, such an approach can be self-indulgent and historically impoverishing. There is little social context for the individuals discussed, little consideration of the wider professional concerns involved in the emergence of neurology as a speciality, and little attempt to integrate Spillane's principal figures into mainstream medical history. Furthermore, the focus is too rigidly Anglo-American, with occasional excursions into France but little on the equally important German scene. M.H. Romberg gets some consideration, probably because his book was translated into English, but the earlier work of men such as K.F. Burdach, and the rich neurological and neuropsychiatric tradition created by Monakow, Westphal, Korsakoff, Benedikt, Wernicke and others are essentially neglected. It is also historically distracting to concentrate on organic diseases of the nervous system at the expense of "functional" disorders, hysteria for example, which so preoccupied physicians such as Weir Mitchell and Russell Reynolds. Aphasia and the neurological consequences of tertiary syphilis also get curiously brief attention.

It may seem ungenerous to carp at what Dr Spillane has failed to give us, when he has given us so much. For his book is a pleasure to read, and the copious use of quotation gives it the flavour of a sourcebook in the history of his discipline. It distils the secondary literature on many pioneers in neurology and the neurosciences. But it is decidedly chapters in, rather than a history of, neurology.

#### Ecology: much to be done in the North

Stanwyn G. Shetler

The Boreal Ecosystem. By James A. Larsen. Pp.500. ISBN 0-12-436880-8. (Academic: 1981.) \$45, £29.80.

ECOLOGICAL studies of the boreal forest region have proliferated in the past few decades, and synthesis of them is certainly overdue. Larsen's impressive and scholarly treatise is not all we might have hoped for, but it will be welcomed eagerly by all boreal biologists and allied students of the North.

The book proceeds from a consideration of the glacial-postglacial history of boreal vegetation to discuss soils, climate, plant communities, various processes, and the economic utilization and management of the boreal forest ecosystem. Although a substantial portion is devoted to presenting some of the author's own data from his 20 summers of fieldwork, overall the book is a review and analysis of other published works.

Despite the ambitious title, the work is less than a definitive treatise on the boreal forest biome. This is not a synthesis for animal ecologists - except for one interesting but general chapter on animal populations ("The Trophic Pyramid"), The Boreal Ecosystem deals with plants. Geographically, the author confines his treatment largely to North America, with the Canadian sector, where he has had the most experience, receiving the most detailed consideration. Eurasia, with twothirds of the world's boreal forest area, is compared briefly here and there, and the extensive Scandinavian and Russian boreal ecological literature is only lightly covered. The perfunctory paragraph on the Appalachian extension of the boreal forest, which long has intrigued American ecologists, is disappointing.

The study of boreal ecology, as ecology generally, often has been hindered by differences in method and terminology. Fortunately, Larsen wastes no time in equating "boreal forest" in the American sense with "taiga" in the Russian sense; thus he does not perpetuate the erroneous notion commonly encountered among North American botanists that taiga is forest-tundra. Probably no two ecologists use the term "muskeg" in precisely the same sense, and such pivotal terms as this, which the author uses throughout, should have been more explicitly defined at the outset.

Larsen, a modern quantitative plant ecologist, begins and ends with an expression of faith in modelling and systems analysis, and he never misses a chance to demonstrate the superiority of the continuum concept and method in analysing boreal forest communities. Continua do not reduce easily to definable systems, however. If he does anything with his wealth of data and description, he convinces us that there are many boreal forests

and environments — in short, many ecosystems, which form a baffling continuum from the tundra complexes in the north to the temperate complexes in the south. Though he drops generalizations here and there throughout the book, most never quite jell, and, while remaining optimistic about future breakthroughs, he is the first to concede that we have not begun to know how to elaborate a workable model or to put numbers into the equations.

We cannot hold him accountable for the untidy nature of ecological science, and we can applaud his quest for the grand synthesis. But Larsen never quite rises above description, in spite of his ecosystem point of view and emphasis on dynamics and process. Nonetheless, he does bring many fresh insights and promising quantitative methods to bear on some of the most fascinating but perplexing and intractable ecological riddles of the North: vegetation history, northern forest limit, nature of succession, permafrost, broad-scale vegetational uniformity masking great local variation, to name a few. His analyses of the forest boundary communities and environmental conditions (particularly the frontal air mass dynamics) and of the inadequacies of classic successional concepts are especially useful and enlightening.

Much of the book is too detailed and heavy-going for quick and easy comprehension, and Larsen's treatise comes off more as a compilation and review than as a challenging new synthesis. Nevertheless, as a compendium on the state of knowledge of the North American boreal forest and its ecotones to the north and south, this unique book is the best and most complete single volume available.

Stanwyn G. Shetler is a Curator of the Department of Botany at the Smithsonian Institution, Washington DC.

#### **Evolution writ small**

R.P. Ambler

Biochemical Evolution. Edited by H. Gutfreund. Pp.368. ISBN hbk 0-521-23549-9; ISBN pbk 0-521-28025-7. (Cambridge University Press: 1981.) Hbk £30, \$69.95; pbk £12.50, \$24.95.

THERE are two ways in which evolution can be studied at the biochemical level. One is by the traditional comparative approach, epitomized 40 years ago by Baldwin's An Introduction to Comparative Biochemistry (Cambridge University Press, 1940). The other is through molecular

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genetics, following changes in gene sequence, number and position, while speculating about the accompanying effects on gene products, and studying functional change through enzymology or population genetics.

These approaches are illustrated by the nine essays that make up the present volume. Pink's excellent chapters on the major histocompatibility antigens and on immunoglobulins illustrate the power of modern molecular genetics in systems where comparative information is very limited. Pink is well aware of the need to consider function in evolving macromolecules, although in the immunoglobulins relevant information is limited. Is it even necessary (p.249) to accept that V gene proliferation is under selective control? In contrast, Crecitelli's account of the vertebrate visual pigments has very little to do with evolution. Weeds and Wagner's chapter on muscle contraction considers Kretsinger's and Collins' speculations about the universality of the structure of the calcium-binding site in contractile and other proteins, but only touches on the question of the multiplicity of actin and myosin genes.

Rao, Hall and Cammack illustrate some of the weaknesses of the comparative approach in their chapter on the photosynthetic apparatus, in which the now conventional attempt is made to derive from a synthesis of the properties of present-day organisms a synoptic account of the history of metabolism on Earth. In contrast, Clarke, in an essay that has a wider scope than its title "Enzymes in Bacterial Populations" suggests, is wise and sceptical, for example in pointing out that early metabolic pathways, like organisms, may have become extinct. In her final sentence she emphasizes the speed with which microbial evolution is observed to occur, a paradox when contrasted with speculation that particular present-day bacteria are recognizably similar to pre-Cambrian organisms.

Peacocke contributes a useful chapter on methods of data handling for phylogenetic trees. The longest section in the book is from Schuster, who considers various aspects of prebiotic evolution. After a long explanation of hypercycles, he concludes that

many plausible models (for the origin of the genetic code and the formation of protocells) can be proposed, but it is very hard to decide between them or even to suggest experiments to prove or disprove them.

Overall, the book suffers from insufficient editorial guidance on chapter content and emphasis, and the introductory chapter by Gutfreund does little to link the rest of the book together or to point the way of future experiment or speculation.

R.P. Ambler is a Reader in Molecular Biology at Edinburgh University.

## All angles on contemporary cosmology

Bernard J.T. Jones

The Isotropic Universe. Monographs on Astronomical Subjects, 7. By D.J. Raine. Pp.253. ISBN 0-85274-370-X. (Adam Hilger, Bristol/Heyden, Philadelphia: 1981.) £19.50, \$49.

EDWIN Hubble made a number of discoveries of fundamental importance in establishing our picture of the Universe, foremost amongst which was the realization that the Universe is expanding. On the simplest level of interpretation this astonishing fact demanded an origin for the Universe at a finite time in our past — a "big bang". Of almost equal importance was the realization that the Universe looks the same at great distances in whatever direction one observes: this homogeneity and isotropy fitted in well with the simple cosmological models deduced by Friedman and Lemaître from Einstein's general theory of relativity. These observations led to one of the great controversies of modern science: the issue was whether the Universe really had a singular origin (the big bang theory), or whether matter was being continuously created so as to make up the loss due to the expansion (the steady-state theory). In 1965 the debate was resolved with the discovery of the cosmic microwave background radiation by Penzias and Wilson. This discovery established the idea of a hot, singular origin for the Universe, and subsequent measurements affirmed the isotropy of the Universe to better than one part in 104.

Cosmology thus became a physical rather than philosophical science, and with this change of emphasis came new questions. Why is the Universe so isotropic? Was it born that way, or did physical processes conspire to create the present isotropy? How did the present observed structure (galaxies and galaxy clusters) originate? What was the nature of the cosmic singularity?

These are the complex and often controversial issues which Derek Raine presents in this book without submerging the reader in a maze of equations and concepts from differential geometry. Of course, to go beyond a simple description of the issues requires more in the way of mathematical hardware, but that step is generally covered by adequate references to the more specialist literature. The discussions of "bang" and "whimper" singularities, and of rotating universes are particularly well done at this pedagogical level, though there are a few places where the going is quite hard (the Bianchi classification of homogeneous three spaces is perhaps the most challenging concept in the book).

Mach's principle, the "anthropic principle", the isotropy of the Universe and the arrow of time are all mentioned and put in context (but for some reason there is

no reference to Paul Davies' book *The Physics of Time Asymmetry*, published by Surrey University Press in 1974).

The Isotropic Universe is not confined to issues arising out of general relativity and the wish to explain space-time structure, though these are certainly the strongest and best-organized sections. The table of contents reads like a check-list of 95 topics every enthusiastic cosmologist should know something about, whatever his or her speciality. With a mere 250 pages in the book some topics get a rather brief treatment, though Raine succeeds in hardly ever lapsing into a "lecture-notes summary" style and maintains a consistent level of pedagogy. It is the pedagogical style that is, in my opinion, the book's strongest point. The discussion of the use of correlation functions and power spectra in studying the clustering of galaxies is a noteworthy example of this. As is often the case in teaching this subject, the mathematics becomes fairly complex for several pages. However, there are some good examples of the use of correlation functions and the two-dimensional power spectrum is treated via a projection of the sphere, thus eliminating the use of spherical harmonics.

So, who might benefit by reading this book? Although the author describes it as

Peaceful Uses of Nuclear Explosives

Lynn E. Weaver, Editor 348 pages, \$8.50 ANS Order No. 410002

Nuclear explosives engineering education, from the technologies required to curriculum development. The theme was organized in five sections dealing with: (1) status of nuclear explosives engineering in 1969, (2) technological requirements of nuclear explosives engineering, (3) legal problems and educational programs, (4) university research and man-

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power needs, and (5) educa-

an intermediate-level introduction, the unaided physics or mathematics graduate may have difficulty in many places owing to a lack of sufficiently diversified background knowledge. The professional mathematician or physicist should experience less trouble in this regard. However, as a teaching book for a graduate course, its style and organization are excellent. My major concern in this respect

lies in what I would regard as an aberrant choice of units (consistency with other noted texts in this field would have been most welcome) and a price that virtually excludes all but the most enthusiastic students.

Bernard Jones is at the Institute of Astronomy, Cambridge, and a Lecturer in the Department of Applied Mathematics and Theoretical Physics, University of Cambridge.

#### Molecular stickiness and its consequences

J.M. Creeth

Protein-Protein Interactions. Edited by C. Frieden and L.W. Nichol. Pp. 403. ISBN 0-471-04979-4. (Wiley: 1981.) £36.95, \$66.45.

THE past year has seen the publication of several quite advanced and thoughtprovoking texts in physical biochemistry -Spragg's The Physical Behaviour of Macromolecules with Biological Functions, Cantor and Schimmel's multivolume Biophysical Chemistry and Richards's more physically-inclined Introduction to Physical Properties of Large Molecules in Solution spring immediately to mind. Now we have Protein-Protein Interactions, and one should say immediately that it overlaps but little with the more general texts. It is a book which will satisfy the specialist, although the general biochemical reader might find it rather hard going in parts. Its main function will probably be to provide a point of take-off for those research workers facing a suspected problem in protein interaction, and in this capacity it should be excellent (although it must be noted that gel-forming protein systems are not included).

The range of topics covers both the essential ground work of elucidating protein structure and behaviour in solution two excellent chapters, these, with much of the mathematics compressed into an appendix - together with quite detailed surveys of the methods currently accepted as most useful. Winzor covers massmigration (including its many recent refinements), and there is an interesting account by Cox of the successful computer simulation of sedimentation velocity. Jeffrey's chapter on equilibrium methods deals mostly with sedimentation equilibrium, as one would expect from the vast amount of work done in the past decade. Although the methods may be used to analyse associations — even if complicated by heterogeneity, non-ideality and pressure-dependence of specific volumes - often, as is made plain, the unambiguous solution is singularly hard to obtain.

Hammes describes the extremely sensitive fluorescence methods and Frieden the more specialized techniques available

for interactions involving enzymes. Timasheff discusses the topical problems inherent in the self-assembly of rod- and tube-like polymers, and a final chapter by Nichol and Winzor deals with the profound questions of how biological control mechanisms can arise through interactions among macromolecules and between them and small molecules. A measure of the practical application of what may appear to be an abstract development is the inclusion here of a section on lymphocyte activation.

Whether physical biochemistry exists (or should do so) as a separate discipline has recently, and quite rightly, been questioned. Although many of us would prefer to minimize the distinctions, conscientious readers of this book will be left in no doubt as to the vitality of the discipline, and the readiness of its practitioners to tackle problems in the main stream of biology.

Michael Creeth is on the MRC External Staff in the Department of Medicine, University of Bristol.

#### Potential reference

Paul Barnes

Intermolecular Forces: Their Origin and Determination. By G.C. Maitland, M. Rigby, E.B. Smith and W.A. Wakeham. Pp.616. ISBN 0-19-856611-X. (Clarendon/Oxford University Press: 1981.) £39.50, \$69.

THE four contributors to this work have clearly striven to produce a definitive reference text on the title-subject. The authors pay due respect to the classic treatise of J.O. Hirschfelder, C.F. Curtiss and R.B. Bird (*Molecular Theory of Gases and Liquids*; Wiley, 1954) but, rightly, also point out the need for a review of developments of the past quarter century.

First, they cover the historical perspective and basic statistical thermodynamic theory, then go on to discuss the relevant experimental techniques of molecular beam scattering, properties of gases and spectroscopy. Throughout this latter part the authors mostly keep to their main task of elucidating the intermolecular potential from the experimental data. The application to condensed phase (solid/liquid) potentials is reserved for Chapter 8, prior to the final summary (Chapter 9).

The format of the book is distinctly "reference style". There are the inevitable inhomogeneities in style and a degree of repetition, but the book is not intended to be read straight through; indeed, the authors are to be congratulated on making each section self-sufficient and, with this in mind, reading schemes are included. The extensive use of valuable appendices strengthens this approach, though even more use could have been made of them in places, for example for the lengthy quantum-mechanical derivations and scattering theory in Chapter 4 on molecular collisions.

The general approach to the subject is one of caution and commendable rigour. The authors continually try to point out the lessons to be learnt from the past (for example, the excessive use of oversimplistic potentials, neglect of pairwise non-additivity, fitting of potentials to inaccurate experimental data) and to define clearly the status of current research on potentials. The deliberate omission of hydrogen-bonding and ionic interactions is a key factor — this allows them to maintain their rigour throughout the book, though in doing so it could rob them of a larger "potential" readership.

This book will be well suited for those wishing to gain a good grounding in the use and elucidation of intermolecular potentials for the simpler systems, and also for those who may need to extract just part of that wealth of detail. Among such people will be computer simulation workers who, incidentally, may feel that the debt of the subject to computer simulation (or numerical quantummechanical calculations) has not been fully acknowledged; only three pages are directly devoted to computer simulation (in Chapter 8 on condensed phases), yet it can be argued that the development of computing is a primary reason for the advances made since the work of Hirschfelder, Curtiss and Bird, and also represents one direction where many further advances are to be expected. For all this, Chapters 7,8 and 9 (on spectroscopic measurements, condensed phases, and our present understanding of intermolecular forces) are particularly enjoyable and are recommended for inclusion in a "first read".

Despite the somewhat cautious approach and the limited range of bonding species discussed, it is to be hoped that many readers will sample this worthy book.

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#### **Biochemicals**

# The increasing cost of consumables

THE Medical Research Council of the United Kingdom, in its annual report for 1980-81 published two weeks ago, drew attention to the consequences for its support of research projects of the increasing costs of consumables. In reality, the council appears to have relatively little to complain of, for it was apparently able to persuade the Advisory Board for the Research Councils (which advises the Department of Education and Science on the division of research funds between the research councils) that some allowance should be made for the way in which the costs of consumables have increased more quickly than retail price indices in the past few years. Understandably, however, there are fears for the future, and not merely in Britain. When all grant-making agencies' budgets are stagnant (or falling), is there a danger that research programmes will be cramped for want of the funds with which to buy consumables?

The simple answer is yes; in the future as in the past, the lack of petty cash (or the authority to sign order forms) will prevent some investigators from doing what they wish. The more important question is whether the investigators that matter, those likely to break new ground, will be hampered in their work. There, most probably, the answer is no. There are good reasons why the fields in which the cost of consumables is most onerous are also novel fields, which most grant-making agencies are likely to have backed with some enthusiam and thus generosity.

#### Recombinant DNA

Thus it is that recombinant DNA research has become, a little to its practitioners' surprise, one of the fields in which the cost of consumables has become most conspicuous. One unit working in the field now reckons to spend roughly a third of its total costs (salaries, but not heat, light and general overheads) on consumables. Radioactively-labelled compounds and restriction enzymes are the chief cost, with fetal calf serum (at about £100 a litre) a steady drain on the budget because the laboratory is also involved with culturing animal cells. The unit reckons that it would be spending even more on consumables if it bought in, as it might, regular supplies of dinucleotides and perhaps even customsynthesized oligonucleotides - a milligramme of a 14-unit oligonucleotide synthesized to order will typically cost \$4,000.

Part of the explanation of these high costs is nevertheless readily understood—and widely accepted by potential customers. In this field as in others in the past, the materials that laboratories buy from suppliers include many which would,

until recently, have been made in the laboratory by the research people themselves. Synthetic oligonucleotides, used for example as probes for particular DNA structures within genes or as building blocks for the synthesis of larger pieces of genetic material, are a good illustration. Indeed, many laboratories prefer, at least for the time being, to make such materials for themselves on the grounds that, in that way, they can be sure that they have synthesized the intended product. But the skilled time required can be substantial.

#### Why buy?

Thus the alternative of buying in from a commercial company is in essence a means of augmenting a laboratory's manpower. Moreover, even at the prices at which materials are supplied, buying-in may be entirely economic when account is taken not merely of the salaries of those who would be occupied with in-house manufacture but of the way in which senior people would be diverted from more creative work by the need to synthesize, or supervise the synthesis of, straightforward chemicals. The old proverb "Penny wise, pound foolish" applies, even though all grant-making bodies may not yet agree.

These circumstances are neither novel nor unfamiliar. Most types of scientific instruments, for example, have usually begun their existence as custom-built machines developed by a single investigator. Even when commercial machines appear on the market, some investigators prefer to make their own versions, partly for the sake of extra sensitivity or flexibility. The point at which a laboratory decides to rely on commercial instruments must necessarily vary from one place to another. One telling consideration is usually the question of when academic laboratories can no longer match the engineering quality of commercial instruments. But there are frequent examples, perhaps more common in the physical sciences, where commercial versions of valuable hand-built equipment never appear, with the result that investigators must reconcile themselves to remaining partly instrument engineers.

One of the reasons for some of the confusion in the commercial supply of chemicals used in recombinant DNA research is linked with the rapid growth of interest in the field at laboratories not previously involved. At the same time, the widespread sense that the recombinant DNA field offers opportunities for entrepreneurs seems to have stimulated the formation of a number of small commercial organizations offering to supply a comprehensive list of materials in some narrow field — dinucleotides and

oligonucleotides for example. As things are, one man, a few technicians and an automatic nucleotide coupler may constitute a business.

Classically, the economists would predict that these circumstances would first stimulate competition, driving prices down. But it is by no means clear whether the market is sufficiently price-sensitive to have this effect. Another possibility is that the newer smaller companies will find it convenient to operate under the wings of larger and well-established concerns more skilled at distribution but anxious (as always) to supply as wide a range of biochemical materials as possible.

Signs of change are already under way in the market for monoclonal antibodies, the most immediate potential source of income for the host of genetic engineering companies set up in the past few years. Eventually, no doubt, some monoclonal antibodies will turn out to have a substantial market, perhaps in the purification of interferon or other proteins with a large potential usefulness in the pharmaceutical industry.

No doubt the market for these products will be determined internationally by means of competition between the suppliers. More specialized antibodies, used only in research, are likely always to be more highly priced. Their suppliers will, however, always be well aware that users in research laboratories can make their own - and will not be prevented from doing so by whatever patent protection applies to the commercial use of the materials. Moreover, many small suppliers must be acutely aware that their costs do not rise linearly with the quantities of these materials they can sell. Competition and sales apparently account for the quite sharp reduction of the prices of monoclonal antibodies in the past few months.

The prospect, then, is that the present high cost of the specialized biochemicals that recombinant DNA research has foisted on itself may well be abated as the years or even months go by. Their places at the top of the lists of expensive biochemicals will no doubt be taken by other materials whose importance is not yet apparent — and which may not yet exist.

#### No saving

Whatever happens to the cost of individual biochemicals, however, there seems no doubt that the cost of consumables in biochemically inclined laboratories will continue to increase. Several laboratory managers report that in the past few years they have been spending increasing amounts on restriction enzymes as increasing numbers of research groups take an

interest in recombinant DNA research. In real terms, the unit costs of these materials seem not to have risen in the past year or so, although they appear also not to have fallen as increased usage might have made possible.

Other natural products are a less predictable burden for laboratory accountants. The recent vagaries of the market in fetal calf serum seem to have been a particular source of trouble and for a variety of obscure reasons.

Fetal calf serum is an essential ingredient of much tissue culture work, but for reasons that remain obscure. The material itself is a by-product of abattoirs, and its supply thus depends to some extent on farmers' willingness to see their cows slaughtered while carrying calves, which is in turn a function of their estimate of the future trend of agricultural prices.

Twelve months or so ago, supplies of fetal calf serum were exceedingly hard to come by, and also expensive — at the peak of the market, the price exceeded £150 a litre. The explanation seems to have been that a fluctuation in the supply of fetal calves coincided with the decision of several commercial companies to produce substantial quantities of interferon from cell lines cultured in the presence of fetal calf serum.

#### Limited choice

At the height of the shortage, investigators were perhaps less distressed by the price of fetal calf serum than by suppliers' suspension of their previous practice of supplying samples from several batches of serum so as to allow the choice of a batch found suitable for intended tissue cultures. Although this practice has now been restored, and many laboratories have also found ways of economizing in their physical use of fetal serum - either by using less of it or by substituting other materials (such as newborn or even adult bovine serum) - anxiety persists that demand for material may eventually permanently overtake the natural supply. The question naturally arises whether the genetic manipulators should not now set out to help their colleagues in tissue culture laboratories by identifying the essential ingredients of fetal calf serum and, if need be, making them artificially.

The third important area of spending on consumables that seems to laboratory managers a heavier burden now than previously is the purchase of radiochemicals. As with restriction enzymes, the underlying difficulty is that laboratories are now using more of these inherently expensive materials, and in a greater variety of formulations.

The cost of radiochemicals is to a large extent determined by the salaries of those who make them. And even though there has been in the past few years a substantial growth of the demand for certain materials, small batch-production is still necessary for most radiochemicals.

Interest in recombinant DNA techniques has for example enormously stimulated the consumption of nucleotides labelled with <sup>32</sup>P.But with its short half-life of 14 days, and the usual problems of chemical degradation caused by radioactive decay, it is still necessary to supply batches of material against firm advance orders.

The manufacturers are shy of saying how much new business has been stimulated by recombinant DNA research, although some estimate that the demand for some of the more widely used radiochemicals is doubling every few months. Both the principal suppliers (Amersham International and New England Nuclear) are seeking to meet the needs of their new customers by making up radiochemicals in more convenient formulations — labelled nucleotides, for example, may be supplied in aqueous solution (thus avoiding one tedious preliminary step).

The trend towards the supply of radiochemicals with greater specific activity has also continued. Ultimately, radiochemicals of all kinds are used in some form of assay. the sensitivity of which depends inherently on the fraction of atoms at labelled site which are radioactive. Some of the <sup>32</sup>P nucleotides now available have a specific activity that is more than half the theoretical maximum, implying that more than half of the nominated phosphorus atoms consist of <sup>32</sup>P. Superficially, these materials are not disproportionately more expensive than others with less specific activity. To investigators, enhanced sensitivity and the opportunity to use smaller quantities of material are advantages that probably always outweigh the extra cost.

Even so, quite small laboratories using substantial amounts of radiochemicals consider it well worthwhile to make special deals with the suppliers. The usual arrangement is that a laboratory will undertake to buy during a year radiochemicals of more than some specified value, winning a discount on catalogue prices in return.

Although laboratories which are parties to such special arrangements are usually fiercely proud of having wrung a tough bargain from the suppliers, the arrangements are probably mutually beneficial. In the radiochemicals business, distribution costs are inevitably high, with the result that there must be substantial economies to be won from the repeated use of some well-known distribution route. But there are obvious limits on the extent to which the pooling of business from several laboratories can be used to negotiate even larger discounts from the suppliers of radiochemicals - people need to order their supplies individually and cannot risk the delays, real or imagined, that some central purchasing agency would imply.

So how much does it cost to support with consumables a scientist at the bench? This question, beloved of grant administrators, is now virtually unanswerable. One recombinant DNA laboratory in Britain

estimates that the annual cost of consumables for its particular programme now exceeds £3,500 a year for each working scientist (graduate students and technical assistants included). A similar laboratory in the United States puts the annual cost at "getting on for \$10,000". There is some evidence that larger costs may be incurred in industrial research laboratories working in these expensive fields, no doubt because those working in a commercial setting are more likely to be encouraged to augment their own labour by means of materials bought in from outside.

The relatively high cost of consumables in some fields, however, appears not yet to have much affected the rule of thumb that large laboratories with a diversified programme of research tend to spend about 10 per cent of their total budget (heat and light included) on consumables. Electron microscopists, or those working on X-ray diffraction analysis, can after all get by well enough with photographic film and perhaps a little liquid nitrogen. There is no general rule that capital equipment obviates the need for expensive consumables, as those working with radioimmunoassay know to their cost. Yet it does appear that in any large and diversified laboratory, there will be some investigators whose spending on consumables is for practical purposes negligible.

Many investigators complain, however, that the degree to which the cost of consumables must vary widely from one laboratory to another, according to the nature of the research programme, has not yet been fully appreciated by the grant-making agencies. Moreover, they argue that in the writing of research grant proposals, it is virtually impossible to calculate with any semblance of accuracy the quantities of expensive materials that will be consumed over a period of, say, three years ahead. The agencies, however, have their own internal needs of tidy accounting, although those which are the chief sources of support for research programmes in molecular biology appear to have devised effective ways of functioning flexibly.

#### Supplement to grants

The ideal, from many points of view, would be that grant-making agencies should agree that grant recipients in the fields in which consumable costs are highest should reconcile themselves to regular supplementary requests for funds, perhaps at intervals of six months or so during the tenure of a research grant. At least where university researchers are concerned, there is now little chance that costs can be met from university budgets.

Small research groups such as these are also less able to insulate themselves from the cost of consumables than research laboratories and institutes with their own integrated administration. In such laboratories, administrators seem to be increasingly self-conscious about the costs of consumables, and more eager than ever

to devise ways of making research groups more aware of the high costs that may be involved in their work.

Bulk purchasing seems to be one of the most commonly used devices for keeping expenditure within bounds, and appears to be most effective when applied to what may be called "dry goods" — rubber gloves and small but frequently used items of plastic ware, the cost of which seems to have increased substantially in the past few years. (The common explanation, that plastic materials are oil-based, and that their increased cost reflects the increased international cost of oil, is not all that convincing, for the quantities of hydrocarbon involved are only very small.)

#### **Bulk contracts**

In this spirit, many laboratories appear to have been able to negotiate contracts for the supply of small disposable but widely used items of equipment which offer substantial discounts, sometimes more than 30 per cent, from catalogue prices. Under these arrangements, laboratories may place an order covering twelve months ahead, drawing off supplies as needed from the manufacturer (and thus avoiding the cost of storage in the laboratory and the risk that materials will be lost, damaged or used profligately). Larger laboratories are sometimes able to buy widely used consumable items by asking suppliers to tender for them.

Despite the financial advantages there may be from such arrangements, however, research administrators seem well aware of the limitations of bulk supply. Requiring that orders for standard items of equipment should be channelled through a central office rather than directly from a laboratory to the supplier can cause delay. Moreover, the problems of identifying in advance a laboratory's need of common items of disposable equipment are in themselves formidable. The results may often be uncertain.

There are also limits to the extent to which individual investigators can be persuaded to order biochemicals from some standard supplier, able and willing to supply in bulk. People are rightly unwilling to change from one source of supply to another in the middle of a series of experiments. Equally, there may be frequent occasions when it is necessary to follow some other investigator's recipe for carrying out some experimental procedure.

In the circumstances, laboratory administrators appear to accept, the best hope for economy is that investigators themselves will be conscious of the cost of the materials they propose to use. Those whose research is supported by identifiable grants are encouraged to examine the bills their work generates, signing the invoices personally when this is appropriate. The risk that over-spending may make it necessary to look for extra funds from departmental or institute resources is usually a powerful restraint. The

competing interests of colleagues are more effective spurs to economy than pleas from accountants and managers.

In many laboratories, invoices are centrally scrutinized as a matter of routine, but usually with discretion and tact. Given the wide currency of the impression that the costs of consumable materials have suddenly become onerous, it is surprising that there are apparently few complaints of bumbledom and over-zealous interference.

One of the most effective ways of engendering a sense of economy among investigators, however, is to arrange that they are notionally required to pay even for commodities and services that would normally be provided free of charge. Such a system is operated at the Institute of Cancer Research in London, and covers the provision of services as different as electron microscopy, photography and the supply of laboratory animals.

For practical purposes, close on a fifth of the total cost of the institute's operation is charged out internally in this way. For simplicity, the cost of various units of service is counted in simple standard units — the cost of producing one electron micrograph is reckoned to be very much like that of another. The products of the animal house are reckoned in "mouse units" — the cost of a single mouse. One rat supplied is reckoned the equal of two mouse units, and so on.

#### Self service

Broadly speaking, each of the service departments is required more or less to break even in the course of a year, covering its own salary and equipment costs but not counting general overheads (heat and light) or the cost of management. The result is that the internal services have on the face of things a cost advantage over external suppliers, at least if they operate on a reasonably economical scale. One result has apparently been to persuade investigators to think twice before signing maintenance agreements for equipment which can technically be serviced by the institute's own electronics department.

Both the managers and the users of this system speak well of its effects, psychological though they may be. Investigators apparently welcome the chance to spend their notional money with the service departments, thus avoiding the need to wheedle and cajole people into compliance with their needs.

Such systems for sharing out the internal costs in laboratories have obvious snags, not the least of which is that the supposed arms-length relationship between the service departments and their customers must in practice be modified by a regular exchange of information between the two sides so that the suppliers can make a reasonable attempt to match their supply with the likely demand. It would serve very little purpose if an institute's animal house became a large and efficient producer of laboratory mice if the chief need had

switched from mice to rats.

There is also the obvious danger that such laboratories might become so well used to the convenience of this service arrangement that they cease to be vigilant in calculating their real costs. In the long run, the only effective safeguard is that investigators should be free to buy from outside their own laboratory if the prices there are potentially more attractive. Perhaps the most serious difficulty is that internal service departments are likely to enjoy comparatively low prestige, and thus less ready access to the funds necessary if they are to be properly equipped so as to remain efficient.

Obviously the scope for such arrangements is confined to laboratories with a certain amount of freedom to determine how resources are divided internally. The practice closely follows that in many industrial research and development laboratories, where the cost of supplies drawn from central stores will commonly be used as a management tool for assessing the cost-effectiveness of a research programme.

It is understandable enough that laboratory managers — or at least the more effective among them — should be more proud of the devices by means of which they have encouraged economy among investigators than they are distressed by the cost of consumables. At the same time, it does appear that the area of research in which the cost of consumables has recently sharply increased is probably sufficiently narrow not to have affected the general cost of research.

Objective figures are hard to come by. A document published twice a year in the United Kingdom by the Committee of Vice-Chancellors and Principals, and which purports to provide a breakdown of the general university costs, shows that the cost of a broad category which includes laboratory consumables has, in the past few years, increased at a pace almost identical with that of inflation. But this index, known as the "Brown index" after its original compiler, is probably too crude a measure to reveal the consequences of the increased cost of consumables in those fields in which the pattern of people's research has changed.

One feature of the recently discovered high cost of consumables in some hitherto shoestring fields such as molecular biology is, however, plain. While many grantmaking agencies may have learned to adapt to these high costs, in present circumstances there is very little hope that university laboratories will be able to meet them out of their own departmental allowances. Moreover, the provisions now made for graduate education fall far below the costs likely to be involved. So while there is no direct evidence that the work of active and well-supported groups is at present being restricted, there is a danger that entry into the field may be hampered by the high cost.

## DRRESPONI

#### Continued from page 302

results should the crops be attacked by more than one pest. Chemical pesticides are still the main component of most methods of integrated control and several progressive companies have now developed insecticides (chlorvinphos) that are particularly suitable for this purpose.

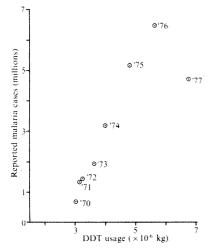
Integrated pest management is now accepted and promoted by WHO and FAO wherever scientific agriculture and progressive health programmes exist. No doubt wider use of this approach is desirable but this depends largely on the availability of well trained specialists and on the understanding by farmers of the need for restraint in the use of chemicals. In this respect the paper by Chapin and Wasserstrom is of some value, although its sensational presentation (with sub-titles such as "Deadly link") undermines the credibility of the authors involved.

L.J. BRUCE-CHWATT Member of WHO Expert Panel on Malaria, London, UK

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Sir — It is generally agreed among malariologists that agricultural insecticides have made a contribution to selection for insecticide resistance in mosquitoes and that such resistance has made a contribution to the resurgence of malaria in Central America and South Asia. It is also generally agreed that one should be very careful before inferring a causal relationship from the discovery of a correlation between two sets of measurements.

No such care was exercised by Chapin and Wasserstrom (Nature 17 September p.181-185). They infer that in El Salvador



"each kilo of insecticide added to the environment will generate 105 new cases of malaria". Taken literally and from their own data this would imply 168 million cases of the disease in a country with a population of 4.3

Chapin and Wasserstrom present three figures with apparently calculated points and

lines but no actual data points. Each graph has a correlation coefficient marked on it of 0.96 or 0.99, but, especially in the case of the curvilinear relationships, the reader has no means of assessing how these coefficients were calculated. Their Fig. 1 is entitled "Effect of DDT on rice production in India, 1970-77" as if the correlation between these two parameters were a simple causal one. In fact, however, it is well known that other independent factors, notably the introduction of high yielding varieties, have both boosted rice yield and allowed and/or required more insecticide usage.

The basis of Chapin and Wasserstrom's curves (Figs 2 and 3) purporting to relate malaria incidence in India during 1969-77 to DDT usage and rice production, is unclear. In the figure (see below) I have plotted the data on malaria incidence issued by the Indian National Malaria Eradication Programme (NMEP) against the data shown by Chapin and Wasserstrom (Fig.4) for DDT usage. Certainly both quantities tended to rise over those years but so did other probably relevant factors such as irrigation. My graph shows a much less startling relationship than Chapin and Wasserstrom's because, according to the NMEP data, the minimum number of cases was higher, the maximum was lower and the malaria resurgence peaked in 1976 (and is reported to have continued to fall in subsequent years). No doubt the NMEP figures greatly under-report the true incidence of the disease, but there seems no reason to suppose that this under-reporting was greater

in the later years than in the earlier: Chanin and Wasserstrom give no information about any "correction factors" which they may have applied to the available data. I conclude that Chapin and Wasserstrom's graphs give a grossly misleading impression that there is a simple causal relationship between agricultural insecticides and malaria.

That the relationship is actually more complex is indicated by the following facts:

- (1) Spraying of cotton crops has the, at least short term, beneficial side effect of suppressing mosquito populations.
- (2) The large tonnage of insecticides used in anti-malaria spraying has certainly contributed to the selection for insecticide resistance in mosquitoes, as shown by the fact that withdrawal of this spraying has been found to lead to a levelling out or decline in the frequency of resistance genes.
- (3) In the Gezira area of intensive agriculture in Sudan, resistance in Anopheles arabiensis is to malathion which is used in anti-malaria spraying and not to the other organophosphates used for spraying the cotton crop.
- (4) Sri Lanka has very wisely banned the use of DDT and malathion in agriculture and reserved them for the anti-malaria campaign but has still had a hard struggle to contain and reverse its resurgent malaria problem.

C.F. CURTIS

Ross Institute, London School of Hygiene and Tropical Medicine, London, UK

#### A reply —

Sir - We are sorry that Professor Bruce-Chwatt disagrees so radically with our interpretation of the facts surrounding malaria resurgence in India. In our own defence, however, we would suggest that the military conflict with Pakistan, the sharp fall in the flow of American aid, the temporary food shortages that took place there between 1973 and 1975, etc., do not explain the abundant entomological reports of Anopheles resistance which we cited in our article - even from such prosperous agricultural regions as Maharashtra and Guiarat. These reports. together with official accounts of the deliberations within WHO and FAO. constitute the major source of evidence upon which we have based our analysis

Finally, like Professor Bruce-Chwatt, we appreciate the difficulties of implementing effective systems of integrated pest management in tropical areas. It was during the successful development of one such system in southern Mexico that the idea of writing this article first occurred to us.

In reply to Dr Curtis, it is unfortunate that in the process of reproducing our illustrations, many of the data points have become difficult to discern. Disregarding for the moment the 1979 figure on malaria incidence in India. however, Dr Curtis's information suggests an even higher correlation between DDT usage and the spread of disease than we have calculated. As for the question of decreased transmission, it may well be true that malaria in India peaked in 1976, but the 1977 figure he cites has been questioned by numerous specialists and in any case does not contradict

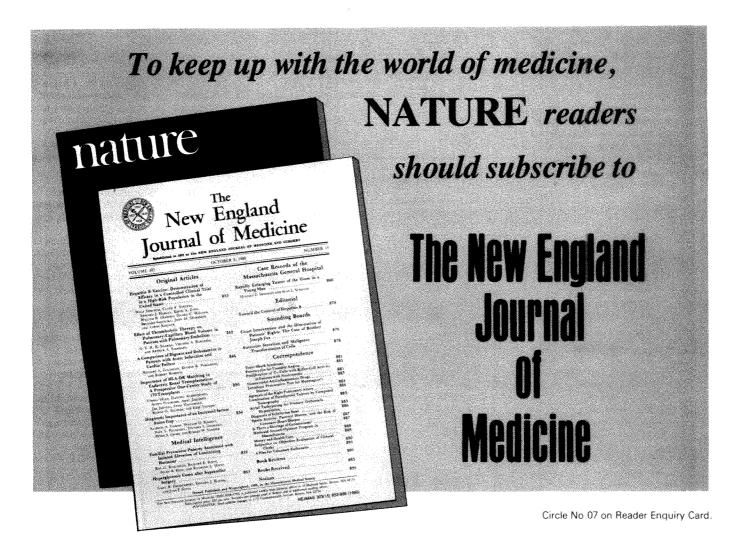
our argument about pesticide abuse.

As for the four points raised in his letter, we offer the following response:

- (1) It is precisely the short-term beneficial effect of insecticides that encourages cotton growers and public health officials to apply them. What we have argued, however, is that the disadvantages of insect resistance soon come to outweigh these rather ephemeral benefits.
- (2) It is difficult to separate the effects of insecticides used for malaria control and those used in agriculture. What is clear, however, is that the decline in resistance after anti-malaria programmes are discontinued is a limited and unfortunately rare phenomenon.
- (3) Although it may be true that Anopheles mosquitoes in Sudan are not resistant to the organophosphates used on cotton, most countries have not been so lucky. How does Dr Curtis explain the almost complete and apparently irreversible resistance among malaria vectors in India, South-East Asia and Central America?
- (4) As the case of Sri Lanka indicates, restricting the use of a particular chemical does not guarantee that mosquitoes will remain susceptible to it: application of related (and even unrelated) compounds is often sufficient to stimulate resistance. Moreover, if initial success in combating malaria leads to the reduction of screening and treatment procedures (as commonly occurs), epidemic resurgence will indeed be exceedingly difficult to control.

ROBERT WASSERSTROM GEORGANNE CHAPIN

Columbia University. New York, New York, USA



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BRL® Gel Electrophoresis Grade N,N'-Methylenebisacrylamide is available as a white recrystallized solid. Acrylic acid content is less than 0.1% and heavy metal content is less than 1.5 ppm to ensure compatibility with nucleic acid and protein work.

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Other gel electrophoresis products include: Sodium Dodecyl Sulfate, Glycine, TEMED, Ammonium Persulfate, Coomassie Brilliant Blue R-250, and Low Melting Point Agarose. All have been specifically prepared and tested for use in gel electrophoresis.

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BRL® Phenol is redistilled and highly purified for the most critical nucleic acid extractions and chromatographic separations. A white crystal in appearance, redistilled phenol is free from interfering levels of heavy metals and inhibitors including H<sub>3</sub>PO<sub>2</sub>. Phenol should be stored at -20°C and is stable for several months. The reagent is packaged in a convenient amber bottle with space provided for the addition of solvent.

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BRL® Cesium Chloride is specifically designed for use in density gradient determinations used routinely in molecular biology and biochemistry. All samples have been checked to ensure that the  $A_{260}$  of a 50% (w/v) solution is zero.

This property becomes important when high purity is required or when relatively less defined systems are fractionated on gradients. This grade of Cesium Chloride can fulfill the needs of most researchers working with virus banding, nucleic acid (DNA, RNA) purification and in studies dealing with nucleoproteins.

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Purity	>99.999%
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BRL also offers high purity Tris, Tris HCl, Urea, Guanidine HCl and Sucrose. All have been prepared to exacting specifications to ensure high purity levels.

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#### **Bovine Serum Albumin**

BRL® Bovine Serum Albumin is acetylated to inactivate contaminating nucleses and proteases.

Each lot of BRL® Bovine Serum Albumin is assayed for exonuclease and phosphatase activity by incubation with 3'- and 5'-end labeled [\$^{32}P\$] DNA. Absence of contaminating endonuclease and ribonuclease is determined by incubation with SV40 Component 1 DNA and ribosomal RNA, and their subsequent analyses by electrophoresis in agarose and denaturing urea gels, respectively. Protease activity is similarly checked by electrophoresis on a 10% SDS-polyacrylamide gel, using ovalbumin and  $\alpha$ -phosphorylase as substrates.

#### Vanadyl Ribonucleoside Complex

Vanadyl Ribonucleoside Complex(VRC) is an effective ribonuclease inhibitor used during cell fractionation and preparation of RNA. Buffers prepared 10 mM in VRC create optimal conditions for ribonuclease inhibition. VRC is supplied as an aqueous solution at a concentration of 200 mM.

#### Isopropylthio-β-D-galactoside

#### 5-Bromo-4-chloro-3-indolylβ-D-galactoside

Circle No. 11 on the Reader Inquiry Card.

# PRODUCT REVIEW

#### Linamarin determination

Now manufactured by BDH Chemicals, Linamarase is a purified enzyme preparation used for determination of linamarin. It has previously proved difficult to distinguish quantitatively between linamarin and free cyanide present in cassava but Linamarase removes this difficulty.

Circle No. 101 on Reader Enquiry Card.

#### Enzymatic diagnostic test

An addition to the ECS range of enzymatic diagnostic test systems is now available from Bio-Rad Laboratories. The ECS uric acid test system depends on a two-stage enzymatic reaction. In the first stage, uric acid is oxidized to allantoin and H<sub>2</sub>O<sub>2</sub>. During the second reaction, the peroxidasemediated oxidative coupling of phenolic dve compounds with 4-aminophenazone results in the formation of a quinoneimine dve, the concentration of which is proportional to the amount of peroxide present and which can be determined by absorbance measurements at 520 mm. Uric acid estimations are valuable indicators in diagnosis of gout and renal dysfunction. Circle No. 102 on Reader Enquiry Card.

#### Hexokinase

SINGLE vial hexokinase is highly specific for glucose, the enzymatic procedure being based on its catalytic action on glucose and ATP, yielding glucose-6-phosphate. Uric acid, ascorbic acid and other substances which interfere with tests dependent on glucose oxidase, do not interfere with this product. The reagent is supplied by Alpkem and can be used with any instrument capable of accurate absorbance readings at 334, 340 or 366 nm. It is highly water soluble and has a stable refrigerated shelf life of up to 18 months.

Circle No. 103 on Reader Enquiry Card.



#### Anti-human fibronectin

AVAILABLE in aliquots of 2 and 10 ml, rabbit anti-human fibronectin antibody is offered by Accurate Chemical & Scientific Corporation.

Circle No. 104 on Reader Enquiry Card.

#### Diagnostic reagents

PREPARED from the Cowan 1 strain of Staphylococcus aureus, the new Staphylococcal Protein A Insoluble reagent from Oxoid is available as a suspension of the heat-killed, formaldehyde-treated cells at a concentration of 25 g dry wt (100 g wet wt) per 1 of buffer. The binding capacity of the reagent for human IgG is 1.5–1.9 mg per ml of suspension. This product is one of the new Oxoid diagnostic reagents range. Circle No. 105 on Reader Enquiry Card.

#### **Scintillation solutions**

LIQUID scintillation counting is described in a brochure from National Diagnostics, which gives details of counting solutions, such as Hydrofluor, intended for use undiluted in aqueous samples. Also included are Dimiscint, which inhibits chemiluminescence, and Autofluor, an autoradiographic image enhancer.

Circle No. 106 on Reader Enquiry Card.

#### **DNA** sequencing vectors

NEWLY available from New England Biolabs as RFI DNA are M13 lac cloning vectors M13mp8 and M13mp9. In addition to the EcoRI, BamHI, AccI, Sall, HincII and PstI sites of M13mp7, these vectors contain Xmal, Smal and HindIII sites. Each of these cloning sites occurs only once within the gene encoding  $\beta$ -galactosidase. Therefore, not only can cloned fragments be selected on the basis of colour on an indicator plate, but insertion of a fragment with two different termini is possible ('forced cloning'). The cloning sites in M13mp8 are aligned in the opposite orientation from those in M13mp9, allowing the orientation of the cloned fragment to be chosen by selecting the appropriate vector.

Circle No. 107 on Reader Enquiry Card.

#### Proteolytic enzyme assay

A PAPER from Varian, 'Assay of proteolytic enzymes using the Varian DMS 90 spectrophotometer, thrombin assay using oligopeptide p-nitroanilide substrates', briefly describes the presence in all living organisms of proteolytic enzymes which function both in metabolism and in complex reactions that modulate the activity of other enzymes.

Circle No. 108 on Reader Enquiry Card.

PRODUCT REVIEW — The product review this week features biochemicals. The notes on these pages are prepared from information provided by the manufacturers. For more details use the reader enquiry card bound inside the journal. The next product review (14 January 1982) deals with water purification and analysis.

#### Bovine serum albumin

ENZYME stabilizer grade bovine serum albumin (BSA; ERT-701) is available from Enzo Biochem. It is free of nucleic acid and protein degrading activities as determined with the appropriate substrates. This product will remain a monomer even after prolonged storage. Enzyme stabilizer grade BSA is supplied in 10-mg quantities at a concentration of 20 mg ml<sup>-1</sup>.

Circle No. 109 on Reader Enquiry Card.



#### **Endotoxin detection**

ENDOTOXINS in water, sterile materials and ingredients for pharmaceutical products can be detected using a new test system from Laboratory Impex. This system detects the presence of Gram-negative endotoxins, is more sensitive than the USP rabbit test and takes only 1 h compared with 24-48 h for the latter method. The reagents required are supplied either in complete kits, with positive, inhibition and negative controls, sterile pipettes and syringes, and labels for identifying the various items, or as 5-ml vials of the lysate and control sets. The Limulus Amebocyte Lysate supplied is reconstituted with the solution being tested and after incubation the presence of endotoxin is indicated by a ring of gelatinized material on the surface of the sample. The test can be used as either a qualitative or quantitative method.

Circle No. 110 on Reader Enquiry Card.

#### Anti-human IgG antisera

FOUR new antisera to human IgG subclasses are now available from Miles: these are antiserum to human IgG1, IgG2, IgG3 and IgG4. Circulating level of IgG subclasses have been associated with autoimmunity, complement-mediated reactions, inhibition of coagulation and numerous other disorders. Antisera to human immunoglobulin subclasses are helpful in classifying monoclonal and polyclonal IgG gammopathies and also hybridomas. A product profile featuring structural diagrams of the four IgG subclasses and product descriptions is available on request.

Circle No. 111 on Reader Enquiry Card.

#### Anti-T lymphocyte

A NEW monoclonal MAS 050 rat IgG antibody against mouse T lymphocytes (clone YBM/29/2.1) is announced by Accurate Chemical & Scientific. This recognizes a monomorphic determinant (Thy-1) on the T lymphocytes of all rat strains. The antibody is secreted by a hybrid cell line produced by the fusion of rat myeloma Y3 Ag 1.2.3 cells with the spleen cells of a rat immunized against mouse bone marrow cells using the technique of Galfre. MAS 050 activates guinea-pig complement and causes lysis of mouse T lymphocytes in a single-stage cytotoxicity test. Its main use is in the separation of mouse T lymphocytes from other cells by complement-mediated lysis, by binding to antibody-coated plastic and fluorescence-activated cell sorting. The antibody can be conjugated directly with fluorochromes or with biotin. It also binds strongly with protein A.

Circle No. 112 on Reader Enquiry Card.

#### **Biologicals**

THE initial product range from Universal Biologicals consists of biologically active peptides from most of the major groups, for example, endorphins, enkephalins, somatostatin and its analogues, bradykinins and parathyroid hormone peptides. Circle No. 113 on Reader Enquiry Card.



#### Serum iron test

NEWLY available from J.T. Baker is a test for serum iron based on the Guanidine/FerroZine method without deproteinization. The test is particularly suitable for automated and semi-automated clinical chemistry instrumentation. To avoid reagent wastage, all components of the serum iron and total iron binding capacity tests are available separately. Major improvements over the conventional Teepol/Bathophenanthroline method are faster reaction time, lower blank values and reduced foaming. In addition, the speed at which lipaemic sera are cleared has been increased.

Circle No. 114 on Reader Enquiry Card.

#### Glycosylated protein test

THE Pierce Glyco-Gel test kit is a research kit for the rapid and reproducible quantitation of glycosylated haemoglobin, glycosylated albumin and non-enzymatic total glycosylated serum protein. The glycosylated proteins are separated by a boronate-agarose affinity chromatography system which is independent of temperature and ionic strength. For glycosylated haemoglobin determinations, c.v. values are typically <2%. The kit contains sufficient pre-packed gel columns, buffers and reagents for 75 quantitative analyses. Circle No. 115 on Reader Enquiry Card.

#### **Electrophoretic markers**

ISOELECTRIC point markers have been introduced by BDH Chemicals. The pl calibration kit (range 4.7–10.6) allows accurate and reproducible measurement of the pH profile of polyacrylamide gels as well as other electrophoretic media such as agarose and other dextran-based polymers. The markers provide a permanent experimental record of the pH profile. Circle No. 116 on Reader Enquiry Card.

#### Platelet control

INTRODUCED by Sarstedt, Inc. as a nonbiological control for testing the accuracy of automatic platelet counters, Thrombo-Control is a suspension of inert plateletsized plastic particles which behave identically to human platelets in all relevant parameters. This material is reported to be stable for up to 1 yr unopened and for 30 days after opening. The suspension is supplied in both a concentrated form for use as a plasma substitute or pre-diluted for automatic platelet counting instruments. The prediluted form eliminates dilution errors. As the product contains no biological material there is no danger of infection.

Circle No. 117 on Reader Enquiry Card.

#### **Interferons**

HUMAN leukocyte interferon (IFN-a; EAB-460) from Enzo Biochem, is produced by normal buffy coat cells induced by Sendai virus and purified by the modified Cantell method to a purity of >106 leukocyte reference units per mg protein. Also available is purified human leukocyte interferon (EAB-451), which is IFN-a (EAB-460) further purified to a specific activity of  $> 2 \times 10^8$  units per mg protein. Both these interferons are calibrated against the NIH international reference standard. Enzo also offers mouse interferon, which is mixture of both IFN- $\alpha$ and IFN-β (EAB-470) prepared in serumfree medium by Newcastle disease virus activation of mouse fibroblasts. Enzo mouse IFN is partially purified and supplied in a sterile lyophilized form, with a specific activity >2 × 10<sup>7</sup> units per mg protein. There is no cytotoxicity on L-929 cells at 105 U ml-1.

Circle No. 118 on Reader Enquiry Card.

# Measuring prostaglandins?

Whether you measure prostaglandins in serum, urine, tissue extracts or even cell culture supernatants, you should know about the Seragen [3H]-RIA kits. Sensitivity, specificity, convenience and reproducibility in prostaglandin measurement are now available in easy to use kits from Seragen.

- · Prostaglandin F<sub>20</sub>
- · Prostaglandin E2
- · Thromboxane B<sub>2</sub>
- · 6-Keto-PGF<sub>10</sub>

High quality antisera to prostaglandins and prostaglandin metabolites are also available.

For further data on the Seragen range of high quality prostaglandin assays and antisera, just call or write to the address below.



SERAGEN, INC., 54 Clayton St., Boston, MA 02122 (617) 265-6004

#### Restriction enzymes

RESTRICTION enzymes and five nucleic acids have been added to the New England Nuclear Nenzymes product line. The restriction enzymes include endodeoxyribonuclease BamHI, endodeoxyribonuclease Bg/II and endodeoxyribonuclease EcoRI. The new nucleic acids are pBR322 (oligo(dG)-tailed) DNA, plasmid DNA molecular weight markers, M13mp7 (oligo(dG)-tailed) DNA, \(\lambda\) DNA (methyldeficient) and exonuclease III (Escherichia coli). Each product is purified to homogeneity as shown by gel electrophoresis and is virtually free from DNA exonuclease, endonuclease, phosphatase and nonspecific protein activities. Also included are DNA/RNA modifying enzymes.

Circle No. 119 on Reader Enquiry Card.

#### Prostaglandin assays

VARIOUS products for immunoassay of prostaglandins have been announced by Seragen Inc. SeraPak immunoassay kits consist of matched components for the radioimmunoassay of prostaglandins  $E_2$  and  $F_{2a}$ . Each kit contains sufficient reagents for 200 assay tubes. SerAb antibodies are high quality, specific antisera for immunoassay of PGE<sub>2</sub>, PGF<sub>2a</sub>, 6-keto-PGF<sub>1a</sub>, PGD<sub>2</sub> and thromboxane B<sub>2</sub>.

Circle No. 120 on Reader Enquiry Card.

#### Isoelectric marker

A SINGLE vial method to mark polyacrylamide gels for isoelectric separations has been devised by United States Biochemical Corporation. Using six cytochrome cs (acetylated), the pl Market Protein Kit may be added with a sample or on a separate gel column as a standard. All six marker proteins are coloured and do not require staining for detection. Complete technical literature is available on request.

Circle No. 121 on Reader Enquiry Card.

#### **Electrophoresis reagents**

A LIST of the main materials used in various electrophoresis methods has been published by Koch-Light Laboratories. The list includes gel components, polymerization initiators and accelerators, buffers, denaturants and reducing agents, dyes and stains, and enzyme detectors. Circle No. 122 on Reader Enquiry Card.

#### **TISSUE CULTURE INCUBATOR**



Modular Incubator Chamber can be used for all types of tissue culture (aerobic, anaerobic). Easy to use! Simply flush with desired gas mixture and place at appropriate temperature. Inexpensive, quantity discounts. billups-rothenberg, inc. pob 977 del mar, ca. 92014-0977 usa (714) 755-3309.

#### Radiochemicals

FOR use in experiments where the presence of potassium acetate is undesirable, L-[35S] methionine (>600 Ci mmol<sup>-1</sup>) from Amersham is supplied in aqueous solution containing 0.1% 2-mercaptoethanol. Also available is [2-<sup>14</sup>C] indomethacin (10-25 mCi mmol<sup>-1</sup>). This a non-steroidal anti-inflammatory drug whch, apart from its major function as a prostaglandin synthetase inhibitor, can also be used in cell cultures to induce synchronous growth, and in membrane studies where its distribution in cells is governed by the pH of intra- and extracellular spaces.

Circle No. 123 on Reader Enquiry Card.

#### Ribonuclease inhibitor

New from Biotec is RNasin, a protein isolated from human placenta tissue which inhibits ribonucleases. This inhibitor helps to maintain the integrity of mRNA, making RNasin useful for cDNA synthesis, in vitro translation, polysome isolation and in vitro transcription. It is stable over a period of months when stored in buffered 50% glycerol solution at -20°C.

Circle No. 124 on Reader Enquiry Card.

#### Chemical catalogue

A NEW chemical catalogue/handbook from Chemical Dynamics, Chemalog 81/82, lists over 8,500 chemicals, biochemicals and reagents and features sections on chromatography, peptides, deuterated solvents, clinical chemicals and optically active compounds. Chemalog 81/82 also includes technical data and references for each item.

Circle No. 125 on Reader Enquiry Card.

#### **HPLC** packing materials

THE range of Nucleosil spherical HPLC packing materials from HPLC technology includes silica, C8, C18, phenyl, CN, NO<sub>2</sub>, NH<sub>2</sub>, N(CH<sub>3</sub>)<sub>2</sub>, OH, cation- and anion-exchange phases. Both 5-µm and 10-um particle sizes are offered. Silicas are available in two pore sizes (50 and 100 Å). A brochure is now available.

Circle No. 126 on Reader Enquiry Card.

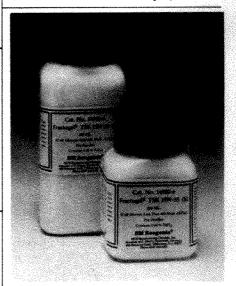
#### Monoclonal antibodies

Six new antibodies to detect cell-surface antigens have been released by Becton Dickinson. These antibodies have a wide variety of applications including indirect immunofluorescence staining, FACS, panning and complement-dependent cytotoxicity. Anti-human Leu 2b is a directly cytotoxic antibody that binds to human T cytotoxic/suppressor cells. Anti-human IgD is specific for IgD in serum and on normal or leukaemic B cells. Anti-mouse Ia.7 (I-E) reacts with specific Ia determinants on mice with  $H-2^{d,k}$  haplotypes. Also available are anti-human Leu 5, antimouse I-Ad and anti-rat x, which is provided as a fluorescein-labelled secondstep reagent for detecting rat antibodies. Circle No. 127 on Reader Enquiry Card.

#### Photoaffinity probes

RECENTLY announced by Schwarz/Mann are two <sup>32</sup>P-labelled photoaffinity probes, [y-<sup>32</sup>P]8-azidoadenosine-5'-triphosphate (<sup>32</sup>P-azido-ATP) and <sup>32</sup>P-8-azidoadenosine-3', 5'-cyclic monophosphate (<sup>32</sup>P-azido-cAMP). These photoaffinity probes are >95% radio-chemically pure by TLC, and are supplied in a methanol solution to increase stability. Non-photoreactive material cannot be detected in these preparations.

Circle No. 128 on Reader Enquiry Card.



#### Gel packing

PACKING material for high performance gel filtration has been announced by MCB. Fractogel TSK is intended particularly for the separation of proteins, enzymes, nucleic acids, oligosaccharides, etc. The packing material consists of a series of hydrophilic semi-rigid spherical gels made from vinyl polymers, designed for lowpressure aqueous gel filtration chromatography. Fractogel TSK will separate sample components in the molecular weight range 100 to 50,000,000 and separations are typically achieved in one quarter the time usually required for conventional soft gels such as dextrans, agarose or polyacrylamides. Fractogel TSK is also stable over a pH range of 1-14 and can be used at pressures up to 100 p.s.i. The gel has a high chemical stability, is resistant to microbial attack and shows minimum gel volume variability during gradient elution. Fractogel TSK is available in 25-40  $\mu$ m and 32-63  $\mu$ m ranges.

Circle No. 129 on Reader Enquiry Card.

#### Phase transfer catalysts

A NEW brochure from Chemical Dynamics Corporation contains a technical discussion of phase transfer catalysis (PTC) theory and practical applications, as well as a complete list of phase transfer catalysts available. Also featured are several new chemicals including prostaglandins, chiral phosphine ligands and peptide reagents.

Circle No. 130 on Reader Enquiry Card.

#### T-cell growth factor

SUPPLIED by Enzo Biochem, human T-cell growth factor (interleukin 2; EAB-451) is produced in concanavalin A-stimulated buffy coat cells. This product maintains the proliferation of mouse and human cells of the T lineage. Human T-cell growth factor is prepared in serum-free minimal essential medium and supplied as a sterile lyophilized powder.

Circle No. 131 on Reader Enquiry Card.

#### Cell culture matrices

New alternative matrices for cell culture are available with a charge surface or a collagen surface. Cytodex 2 and Cytodex 3 from Pharmacia Fine Chemicals are intended for culture of anchorage-dependent cells. Cytodex 2 is a surface-charged bead that is particularly useful for culturing cells of fibroblast-like morphology. Charged groups necessary for cell attachment are present only as a thin layer on the surface of the microcarriers — this gives reduced ion-exchange properties, allowing easy removal of media components for cleaner cell harvesting. Cytodex 3 is a denatured collagen-coated bead, offering a growth matrix more closely approximating the connective tissue surface in vivo. Applications of Cytodex cultures include cellular metabolite response and virus and interferon production.

Circle No. 132 on Reader Enquiry Card.

#### **Electrophoretic blotting**

ADDITIONS to the Bio-Rad line of electrophoresis equipment are the Trans-Blot cell and model 160/1.6 power supply. The Trans-Blot cell is designed to transfer electrophoretically separated DNA, RNA or protein from the gel to an immobilizing matrix in as little as 30 min (compared with 24-48 h for Southern or capillary blotting). Once transferred, the bands are readily accessible for autoradiography, ELISA or fluorescent detection and for preparative elution. The model 160/1.6 power supply has been designed to meet the power requirements of all electrophoretic transfer techniques, with an output of up to 1.6 A essential for the maintenance of a uniform electric field across the large cross-sectional area of the gel.

Circle No. 133 on Reader Enquiry Card.

#### **HPLC** columns

STANDARD 3 Micron Spherisorb columns from Phase Separations generate on average 155,000 plates per m. This, coupled with the fact that it shows negligible decline in efficiency with increasing mobile-phase velocities, makes the 3 Micron Spherisorb column directly applicable to high-speed analysis. Selection of suitable column lengths (5, 10 or 15 cm) with mobile-phase flow rates of 0.5 to 5 ml min<sup>-1</sup> allows maximum flexibility for achieving high resolution and speed.

Circle No. 134 on Reader Enquiry Card.

#### Chromatography matrix

A leaflet describing a new chromatography matrix is available from Bethesda Research Laboratories. RPC-5 Analog matrix has properties similar to those of RPC-5 and is useful for analytical and preparative fractionation of bacterial plasmids and supercoiled viral DNA, cloned DNA fragments and oligomers of ssDNA and RNA, among others. The matrix gives high resolution and allows rapid separations. RPC-5 Analog column chromatography is particularly useful in the purification of plasmids from cellular lysates.

Circle No. 135 on Reader Enquiry Card.

#### **Biological radioiodination**

UNIFORM, non-porous polystyrene beads (Iodo-beads) which have been covalently modified with an oxidizing agent to facilitate the smooth and reproducible iodination of peptides, hormones, proteins and antibodies over a broad range of pH and temperature conditions, are available from Pierce Chemical. To initiate an iodination, Iodo-bead is added to the <sup>125</sup>I-labelling



solution. To terminate the labelling reaction, the bead is removed. Near quantitative labelling with 90-95% protein recovery has been achieved. In addition, the performance of Iodo-beads is not inhibited by azide, detergents, choatrophic agents or high salt.

Circle No. 136 on Reader Enquiry Card.

#### M13 sequencing primer

THE universal M13 sequencing primer, d(TCCCAGTCACGACGT), from New England Biolabs, anneals to the + strand of all M13 lac phages which carry the gene encoding  $\beta$ -galactosidase (M13mp2, mp5, mp7, mp8, mp9 and M13mWJ22). The length and location of annealing of the primer enable reading of the first nucleotide of the inserted fragment while still optimizing the quantity of sequence data obtained from each gel. The 3'-terminal hexanucleotide sequence of this primer was selected because it occurs only once in the M13 genome and assures very low nonspecific priming at other sites on the M13 template.

Circle No. 137 on Reader Enquiry Card.

#### Plant cell enzymes

ENZYMES for use in plant cell biology are now supplied by Genetic Research Instrumentation. These enzymes are Cellulase R10, Pectolyse and Meicelase, and are packaged in 1- or 10-g amounts. This range of plant cell enzymes complements the range of instruments available for plant cell biology, which includes incubators, shakers, freeze dryers, fermenters, centrifuges, laboratory hoods and safety cabinets.

Circle No. 138 on Reader Enquiry Card.

#### **Biochemicals**

PRODUCTS for medical research, from adsorbents and amino acids to venoms and vitamins, are supplied by Koch-Light. The range includes gel permeation media, enzyme immobilization products (Enzacryl), histochemical reagents, hormones, catalysts, carcinogens, nonionic surfactants, nucleosides, buffers and serology reagents.

Circle No. 139 on Reader Enquiry Card.

#### TLC separation medium

A NEW thin-layer chromatography separation medium Fixion, from Chromatronix, differs from other TLC separation media because it separates by ion exchange. Its advantages are high sample throughput, which improves productivity; it handles samples with high salt or acid content; and gives high resolution and efficient separations due to the highly active spherical resin layer. Fixion can be used for one-dimensional separation of amino acids and nucleic acid constituents, determination of essential amino acid content and for the separation of antibiotics, drugs and drug metabolites. Circle No. 140 on Reader Enquiry Card.

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#### Transcription system

A NEW transcription system from New England Nuclear contains a cell-free extract, derived from HeLa cells, which catalyses the synthesis of mRNA precursors when provided with an exogenous DNA containing a promoter for recognition by the polymerase. This reaction is highly sensitive to the presence of  $\alpha$ -amanitin, indicating an RNA polymerase II-dependent reaction. The system is available with either  $[\alpha^{-32}P]$  UTP or  $[\alpha^{32}P]$  GTP for labelling desired RNA transcripts.

Circle No. 141 on Reader Enquiry Card.

#### Ninhydrin reagent

An advanced formula ninhydrin solution (Nin-Sol AF) has been announced by Pierce Chemical. This is an improvement on the popular ninhydrin-DMSO-hydrindantin formula and offers the advantage of better colour response, linearity and stability.

Circle No. 142 on Reader Enquiry Card.

# NATURE CLASSIFIED

LONDON OFFICE Jean Neville 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024)

AMERICAN OFFICES NEW YORK Cathy Moore 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900

PHILADELPHIA Dan Adams (215) 353 6191 ● SAN FRANCISCO JJHS (415) 392 6794
PASADENA JJHS (213) 796 9200 ● DALLAS Roy McDonald (214) 941 4461
HOUSTON Roy McDonald (713) 988 5005 ● FORT LAUDERDALE Brinker & Brinker (305) 771 0064
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#### UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSIOLOGY
Applications are invited for a post of

# POST-DOCTORAL RESEARCH ASSISTANT

to study the effects of reinnervation on motor coordination, using mechanical and electrical recording of muscle.

The post is funded by "Action Research" for a period of two years, and the salary will be in the IA range up to £6.880

Applications, including a full curriculum vitae and the names and addresses of two referees, should be sent to Dr D M Lewis, Medical School, University Walk, Bristol BS8 1TD. (9933)A

#### **BIOCHEMIST**

Tenure track, September, 1982, PhD required. Principal training in Biochemistry, with interest and ability to teach Analytical, Physical (Thermodynamics or Kinetics), Inorganic, or General Chemistry with Quantitative Analysis. Evidence for the following is necessary: potential for excellence in teaching, commitment to the liberal arts, ongoing research plans liberal arts, ongoing research pians and a desire to direct undergraduate research. Initially, principal responsibility will be to teach Biochemistry with laboratory and other upper division chemistry courses commensurate with training. A willingness to help develop a cooperative cell biologybiochemistry program with the Biology Department is important. Department is listed in "Research in Chemistry at Private Undergraduate Colleges" 2nd Ed., Council on Undergraduate Research, 1981.

Send résumé', transcripts of all institutions attended, examples of scholarly work, brief description of research interests, and a discussion of how you might involve undergraduates from a liberal arts institution in your research. Also, arrange for at least three letters of recommendation to be sent to: Dr William J Randall, Chairman, Department of Chemistry, Lewis and Clark College, Portland, Oregon 97219. Closing date for applications is January 15, 1982. Lewis and Clark College is an Equal Opportunity Employer and invites the candidacy of qualified minority persons and women. (NW091)A

INSTITUTE of Cancer Research: A vacancy exists for a Research Officer/Technician in the Institute's Department of Cytogenetics, Royal Marsden Hospital, Fulham Road, SW3. The post is available for two years in the first instance. Current projects in the department include the search for specific chromosome abnormalities in inherited cancers and studies of the action of drugs in vivo and in vitro. Experience in human cytogenetics desirable. Salary grade dependent on age and qualifications. (Research Officer: £5,600 — £7,336 pa) (Technician: £4,958—£6,993 pa) plus London Allowance of £859 pa. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/11. (9962)A

## UNIVERSITY OF THE WITWATERSRAND,

Johannesburg, South Africa

DEPARTMENT OF ZOOLOGY
CHAIR IN ZOOLOGY

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin for appointment to a Chair in Zoology.

It is the University's concern to make an appointment on the basis of outstanding personal attributes rather than field of interest. A particular interest in research, the ability to cross interdisciplinary boundaries and enthusiastic leadership in research will be taken into consideration.

The salary scale attached to this post is  $R20,040\times810$  —  $R20,850\times900$  — R26,250. (£1 = R1.80 approx). Applicants not meeting the requirements for this senior post may be offered appointment at a lower level.

Detailed pamphlets are available on service conditions and the Department of Zoology, and may be obtained from the Secretary, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar (Staffing), University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001, South Africa, with whom applications should be lodged not later than 31 December 1981. (9937)A

# ARMAGH OBSERVATORY Armagh, Northern Ireland

Armagh, Northern Ireland
Applications are invited for the post of

#### RESEARCH ASTRONOMER

tenable from 1st April, 1982

Salary will be at an appropriate point on the University Lecturers' scale £6,070 to £12,860 pa according to qualifications and experience.

Applications stating qualifications, publications, research proposals, and names of three referees should be received not later than 31 January 1982 by the Secretary, Armagh Observatory, Armagh BT61 9DG, N Ireland, from whom further particulars may be obtained. (9969)A

#### UNIVERSITY OF QUEENSLAND Australia

# SHORT TERM LECTURER IN PHARMACOLOGY

Applicants should be experienced in both teaching and research. Preference will be given to applicants whose research interests are compatible with the current research programmes of the Department. Applications close: 15 January 1982.

Salary: \$A19,821 — \$26,037 per annum.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H0PF. (993))A

# nature

has an immediate vacancy in London for an

# ASSISTANT EDITOR

to work primarily on the assessment of physical science manuscripts.

Essential qualifications:

- \* A good science degree
- \* Some knowledge of the earth sciences
- Some research experience
- \* A sense of literacy
- \* Wide general interests.

Applications, including a full curriculum vitae, should be plainly marked "Job application" and addressed to the Editor, Nature, Macmillan Journals, 4 Little Essex Street, London WC2R 3LF to arrive before 15 December 1981. Secondment for not less than two years would be appropriate.

(9927)A

#### ROYAL OBSERVATORY, EDINBURGH Image and Data Processing Unit

# Scientific Officer

There is a vacancy for an Assistant Computer Manager/Software Specialist in the Image and Data Processing Unit at ROE.

The ROE has responsibility for the development and operation of three major national facilities for the support of research in astronomy: these are the 1.2 M UK Schmidt telescope in Australia, the 3.8 M UK infrared telescope in Hawaii and the cosmos high-speed measuring machine in Edinburgh. The ROE is also one of the major nodes in the Starlink Computer Network for astronomical image processing in the UK. There is a strong and active research group with wide ranging interests and there is close collaboration with the University of Edinburgh.

#### DUTIES:

The successful applicant will assist in the management of the computing facilities at the ROE. These computing facilities comprise on-site GEC 4082 and DEC VAX 11/780 mainframe systems plus links to remote central computing facilities. Duties will included the development, documentation and maintenance of scientific applications software, assistance in the efficient running of the computr systems and user liaison.

#### **QUALIFICATIONS AND EXPERIENCE:**

Candidates should possess a degree or equivalent in mathematics or one of the sciences. Some experience of computer systems and programming in a high level language is essential. Experience of hardware and systems software would be desirable but is not essential.

The starting salary will be in the range £5,176 - £6, 964.

Application forms may be obtained from: Mr. R.L. Wison, Personnel Section, Royal Oservatory, Blackford Hill, Edinburgh. EH9 3HJ. Tel: 031-667 3321.

Application forms should be returned by 18 December 1091.

(9968)A

#### MANCHESTER AREA HEALTH AUTHORITY (TEACHING)

#### POST OF DIRECTOR

Christie Hospital & Holt Radium Institute, Paterson Laboratories

To take up post in June 1983 on the retirement of Professor L G Lajtha.

The Director is the Scientific and Aministrative Head of the Paterson Laboratories containing ten Divisions and approximately 180 staff with responsibility to maintain and develop further Research Programmes which are mainly supported by the Cancer Research Campaign.

The Director will be offered an Honorary Professorship in Experimental Oncology at Manchester University.

Appropriate MRC salary scale and Conditions of Service. The NHS or USS Superannuation Scheme will apply.

Job description and further details available from the Area Personnel Officer, Manchester Area Health Authority (Teaching), Mauldeth House, Mauldeth Road West, Chorlton, Manchester M21 2RL (Tel: 061-881 7233 ext 219).

Professor Lajtha will be please to discuss the appointment with intending applicants. Tel: 061-445 8123.

Applications to be returned to the Chairman, Manchester Area Health Authority (Teaching), Mauldeth House, Mauldeth Road West, Choriton, Manchester M21 2RL by 4.1.82. (9942)A

#### DEPARTMENT OF ZOOLOGY University of California, Davis ASSISTANT PROFESSOR

Applications are invited for a tenure-track position at the Assistant Professor level for September 1982. A strong, independent research program is expected; fields of particular interest include, but are not limited to, evolutionary biology, marine or behavioral ecology, and environmental physiology. Preference will be given to candidates able to teach invertebrate zoology at the upper-division level. The appointee may also teach a one — quarter course in introductory biology and will have the opportunity to develop courses and/or seminars in his/her area of expertise.

seminars in his/her area of expertise.

Persons wishing to apply should send a curriculum vitae, reprints of published work, a description of research program and future plans, a summary of teaching experience and capabilities including evaluations if available, and three letters of recommendation to:

Dr. Arthur M. Shapiro, Chairman, Search Committee, Department of Zoology, University of California, Davis, CA 95616.

Final Date for receipt of applications is *February 15, 1982*. The University of California is an equal opportunity/affirmative action employer. (NW101)A

#### RESEARCH POSITION

(C1, Hochschulassistent) in antiinfective immunology available Applicants (PhD or MD) should have a keen interest and experience in antiinfective immunology (T cell cloning, receptor analysis, biochemistry of mediators).

This position allows ample time for research; in addition, participation in teaching lectures and courses in medical microbiology as well as work in the routine laboratory are obligatory. In the latter field, no special experience is required.

For further information contact: Prof. Dr med. Helmut Hahn, Institute für Medizinische Mikrobiologie der Freien Universität Berlin Hindenburgdamm 27, D-1000 Berlin 45, West Germany. (W497)A

# THE INSTITUTE FOR ADVANCED STUDY

will have several openings for members in theoretical physics and astrophysics for the academic year 1982-83. The positions are at a post-doctoral or higher level and applicants will be selected on the basis of their ability to do research in the areas of elementary particles, mathematical physics, astro-physics, plasma physics, general relativity and statistical mechanics. Preference is given to candidates who have received their PhD within the last year or two.

Postdoctoral members frequently collaborate with each other, with faculty members at the Institute or Princeton University, and with researchers at other institutions.

Appointments ae usually for no more than two years and support is typically full salary for postdoctorals and half salary for more senior persons. Women and minorities are encouraged to apply.

For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW1006)A

PROFESSOR. ASSISTANT Biochemistry-Molecular Biology. Tenure-track 12 month position. Candidates should show outstanding potential for research and be interested in team teaching biochemistry and offering a graduate course in proteins and enzymes. Areas of research interest can include (but are not limited to) membrane biochemistry, enzyme regulation or regulation of gene expression. Post-doctoral experience is desirable. This position will be approximately 75% research with an appointment in the Experiment Station. Please send curriculum vitae, a short statement of research interest, and three letters of reference by 15 February 1982 to Dr Wayne E Magee, Head, Department of Bacteriology and Biochemistry, College of Agriculture, University of Idaho, Moscow, Idaho 83843. Affirmative Action/Equal Opportunity Employer. (NW107)A

#### UNIVERSITY OF LEICESTER PHYSICS RESEARCH PhD

The Department of Physics at Leicester offers research opportunities leading to the degree of Doctor of Philosophy in the following areas:

CONDENSED MATTER PHYSICS
IONOSPHERIC PHYSICS
NUCLEAR ELECTRONICS
QUANTUM MOLECULAR
PHYSICS
THEORETICAL PHYSICS

X-RAY ASTRONOMY

The Department has very active, well-balanced research programme which receives a high level of support from both government and industry. In addition to in-house research, collaborative projects are undertaken involving other institutions in the UK and abroad. There are excellent workshop and computing facilities.

Further details and application forms may be obtained from Dr C Norris, Department of Physics, University of Leicester, University Road, Leicester LE17RH. (9948)A

# Readvertisement University of London British Postgraduate Medical Federation CARDIOTHORACIC INSTITUTE CARDIAC MUSCLE RESEARCH UNIT PHYSIOLOGIST/BIOPHYSICIST/ PHARMACOLOGIST (Post-doctoral)

A vacancy exists for a post-doctoral scientist to work on a project utilising intracellular ion-selective microelectrodes in cardiac muscle. Previous experience in electrophysiology would be an advantage. The appointee will work in a multi disciplinary team with extensive and varied research interests relating to the heart.

Salary on appropriate point on Research Assistant Scale 1A, currently £6,070 — £10,575 plus £967 London Weighting Allowance. Universities Superannuation scheme applies.

Further details from Dr Poole-Wilson, (tel: 01-486 3043). Applications with full curriculum vitae, list of publications, names and addresses of three referees to Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP to arrive no later than 4th January 1982.

# UNIVERSITY OF CAMBRIDGE DEPARTMENT OF EARTH SCIENCES RESEARCH ASSISTANT IN STABLE ISOTOPE GEOCHEMISTRY

A graduate or post doctoral research asssistant with a background in mass spectrometry or stable isotope techniques is required to perform a D/H ratio study designed to investigate the possible occurrence in chondritic meteorites of material synthesised by ion molecule reactions in dark interstellar clouds. The appointment is for 2 years with a salary up to £7,700 pa.

Applications including a CV and names of referees to Dr C T Pillinger, Planetary Sciences Unit, Department of Earth Sciences, Cambridge CB2 3EQ, telephone (0223) 355463 ext 283 as soon as possible. (9955)A

#### UNIVERSITY OF PITTSBURGH ASSISTANT PROFESSOR BIOLOGICAL SCIENCES

The Department of Biological Sciences invites applications from highly qualified individuals with a strong research commitment in one or more of the following areas: theoretical ecology, population genetics, evolutionary ecology, physiological ecology, aquatic ecology, microbial ecology or behavioral ecology. A curriculum vitae, list of publications, names of at least three references, and a summary description of your current research program and future plans should be sent to: M Sussman, Chairman, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260. Starting date after July, 1982. Closing date for applications: January 29, 1982.

The University of Pittsburgh, acting in the spirit and the letter of Affirmative Action regulations, actively encourage applications from women, minority members, and other individuals covered by the mandates of Title IX. (NW110)A

#### KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(University of London) Denmark Hill, London SE5 8RX

#### A GRADUATE RESEARCH ASSISTANT

is required to join a team in the Liver Unit, investigating lymphocyte population and suppressor function in children with chronic liver disease. Previous experience in immunology desirable.

The appointment will be for one year in the first instance. Salary on the Research Assistant 1B scale £5,285 — £6,475 plus £967 London Weighting Allowance, according to age, experience and qualifications. Applications, in duplicate, including the names of 2 referees should be sent to the Secretary of the Medical School at the above address, no later than 17 December 1981. (9938)A

#### THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

#### **CHAIRMANSHIP**

Candidates for the chairmanship of the Department of Pharmacology and Toxicology are being sought. Qualifications include an established reputation in research and teaching and the ability to direct a department with an active graduate program and teaching responsibilities for medical, dental, and nursing students.

Applicants should submit curriculum vitae, list of publications, description of research plans, and names of three references by March 1, 1982, to: Dr Dennis J O'Callaghan, Chairman, Search Committee, Department of Microbiology, University of Mississippi Medical Center, 2500 North State Street, Jackson, Miss. 39216. The University of Mississippi Medical Center is an equal opportunity employer, M/F/V/H.

(NW092)A

#### CSIRO

#### **AUSTRALIA**

# **Research Scientist**

(2 Positions)

#### Division of Food Research North Ryde, New South Wales

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,500 employees — 2,700 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

**General:** The research program of the Division includes many aspects of the physical and biological sciences related to the handling, processing and storage of foods and to the needs of the food industry and the consumer.

#### 1. Ref. A2611

Field: Microbiology (microflora of the gut)

**Duties:** To participate in and supervise research on the influence of diet on the microbial flora of the human gut. The study will include the influence of individual foods and food additives and of prior processing of foods on the gut flora and, in turn, the effects of this flora on the intestinal metabolism and subsequent absorption of dietary components.

Qualifications: A Ph.D. degree or equivalent, with experience in the biochemistry and microbiology of the gut microflora, anaerobic techniques, expertise in chromatographic analyses and demonstrable research ability.

#### 2. Ref. A2557

Field: Plant Physiology/Biochemistry

**Duties:** To participate in research program into the regulation of protein synthesis in the cells of mature plant tissues. The program includes the *in vitro* translation of isolated ribonucleic acids, identification by immunological and other techniques of translation products, the cloning of DNA and the measurement of abundances of specific messenger RNAs.

Qualifications: A Ph.D. degree, or equivalent, in Plant Physiology, Biochemistry or other relevant field together with demonstrable research ability. Experience in nucleic acid chemistry and biochemistry is essential and a knowledge of the physiology of ripening and senescence in plants is desirable.

Tenure: Indefinite with superannuation (both positions).

Applications: Stating full personal and professional details, the names of at least two referees, and quoting reference numbers A2611 or A2557, should reach: The Chief, Division of Food Research, CSIRO, PO Box 52, North Ryde, NSW 2113, Australia by 31 December 1981.



#### **Royal Postgraduate Medical School**

(University of London)

Applications are invited for a five-year appointment as

# Lecturer (non-clinical) in the Department of Immunology

A post-doctoral science graduate with experience in immunochemistry is sought. The successful applicant will contribute to the setting up of a postgraduate immunology course. Experience in teaching and organisation of courses is essential. Appropriate technical support will be provided.

There will also be opportunities for research in a well equipped department. The particular interests of the department include major histocompatibility systems, and their relationship to immunologically mediated diseases and organ transplantation, the study of patients with eosinophilia and experimental models of filiariasis. Salary on the scale £6,070 to £12,860 p.a. plus £967 London Allowance.

Applications, enclosing six copies of curriculum vitae and naming three referees, to the Deputy Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W120HS. (01-743 2030 ext. 263) from whom further particulars can be obtained. Closing date 15 January 1982. (9950)A

# **LELUS**

pioneer in bioindustry since 1971, is currently seeking innovative professionals in the following areas:

- Molecular genetics
- Nucleic acid biochemistry
- Molecular virology
- Cloning and expression of eukaryotic genes
- Microbial physiology
- Analytical chemistry
- Enzymology
- -Chemical catalysis
- -Protein chemistry
- -Plant cell genetics
- Biochemical engineering
- Process engineering
- Instrumentation Design

We invite you to submit your resume in confidence to:

> **CETUS CORPORATION BOX NKLH** 600 Bancroft Way Berkeley, CA 94710 (415) 549-3300



(NW086)A

#### ASSOCIATE DEAN FOR **CURRICULUM UNIVERSITY OF MASSACHUSETTS** MEDICAL SCHOOL

Candidate for this position should have a PhD or MD degree. Responsibilities will include the planning of a new curriculum and its ongoing evaluation; should have a strong interest and ability in teaching medical students; should have administrative skills, and be able to relate to large numbers of faculty; will work closely with existing faculty and student committees in the areas of educational policy and curriculum design.

In the organizational structure, the Associate Dean for Curriculum will report to the Academic Dean. The individual will be a member of the Educational Policy Committee and will be responsible for chairing a group of working subcommittees of faculty and students, which subcommittees will design the details of the new curriculum using guidelines recommended by the Educational Policy Committee. The Associate Dean for Curriculum will be responsible for the implementation of this new curriculum including matters such as scheduling, allocation of time to various learning activities, allocation of responsibility to various chairpersons and providing ample liaison and communication with faculty and students. Ongoing evaluation and efficacy should be monitored through contact with the student committee on evaluation of courses, by long term follow-up through feedback from house officer programs, and by any other methods which are appropriate.

This position offers an outstanding opportunity to an individual who wants to make his or her work in the field of medical education, in a school whose excellent faculty is clearly in favor of curriculum

change.

University of Massachusetts Medical Center Interested persons should send résumé to: John P. Howe, III, MD., Vice Chancellor/Academic Dean, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01605.

An Affirmative Action/Equal (NW114)A

#### **UNIVERSITY OF** LIVERPOOL

#### DEPARTMENT OF BIOCHEMISTRY RESEARCH ASSISTANT

Applications are invited from raduates in Biochemistry or Microbiology to work on a project investigating the production of proteinase inhibitors by Streptomyces. The project is funded by the SERC and is under the supervision of Dr R J Beynon.

The appointment will be made as soon as possible at an initial salary of £5,285 pa.

Applications, together with the name of two referees, should be received not later than 14th December, 1981, by The Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/906/N. (9936)A

#### KING'S COLLEGE HOSPITAL MEDICAL **SCHOOL** (University of London)

Denmark Hill, London SE5 8RX

#### A POST-DOCTORAL RESEARCH ASSISTANT

is required to join a team in the Liver Unit, investigating lymphocyte cytoxicity to hepatocytes in the pathogenesis of chronic liver disease in children and adults. Experience in lymphocyte culture techniques and immunofluorescene would be an advantage

The appointment will be for one year in the first instance. Salary on the 1A scale £6,070 — £7,290 plus £967 London Weighting Allowance, according to age, experience and qualifications. Applications, in duplicate, including the names of 2 referees should be sent to the Secretary of the Medical School at the above address, no later than 17 December 1981. (9940)A

#### ST MARY'S HOSPITAL MEDICAL SCHOOL (University of London) Norfolk Place, London W2 1PG

#### DEPARTMENT OF VIROLOGY RESEARCH ASSISTANT OR MEDICAL LABORATORY

SCIENTIFIC OFFICER

required for a grant-funded, temporary position for one year to assist in program of comparative analysis of poxvirus genone structure and function. Applicants should be familiar with DNA technology relevant to restriction analysis and experience in tissue culture/Animal virology techniques would be advantageous. Appointment will start January 1982 or soonest and salary will be appropriate to qualifications.

Enquiries to Dr L Archard (01) 723 1252 ext 236. Applications in form of full cv and names and addresses of two to Assistant Secretary (Personnel) at above address not later than 14.12.81. Please quote Ref: RA/LA. (9963)A

#### The Flinders University of South Africa School of Physical Sciences

Applications are invited from persons holding a PhD degree in chemistry, physics or ceramics engineering for appointment as:

#### Research Fellow/ Associate in the **Ceramics Laboratory**

The appointee will investigate the chemical physics of interfacial reactions between noble metals and ceramic oxides. Emphasis is placed on impedance spectroscopy of faradaic charge transfers and phases, electron and optical microscopy, and characterisation of reaction products for thin noble metal films on single crystal oxide subtrates.

Available equipment includes a Solartron FRA with interface, a MINC 11/23 computer, an extensive range of electrochemical equipment for AC and DC measurements and access to Prime 750 and DEC system 10 computers. The Group is well supported by engineering and electronic workshops.

The appointment will be for one year in the first instance. Salary range \$A17,083 — \$A19,570, depending on qualifications and experience.

Further information may be obtained by contacting Associate Professor H. J. De Bruin (telephone 08-275 2191: telex 89624 Flindu).

Applications including a curriculum vitae with full details of qualifications and experience and the names of three referees of whom confidential enquiries may be made, should be lodged with the Registrar, The Flinders University of South Australia, Bedford Park, S.A. 5042 Australia, by 4 January, 1982. (9944)A

#### UNIVERSITY OF GLASGOW

DEPARTMENT OF MATERIA MEDICA, STOBHILL GENERAL HOSPITAL, GLASGOW RESEARCH ASSISTANT

There is a vacancy from 1st January, 1982 for a Research Assistant to join a pharmacological project investigating the role of peptide neurotransmitters — enkephalins, angiotensin and Substance P — in central regulation of baroreceptor and chemoreceptor reflex function. A background in pharmacology or related neuroscience is desirable. The post is available for 18 months and is supported by the Scottish Home and Health Department; starting date by arrangement. Salary will be within £6,070 to £7,290 on Range 1A of the scales for Research and Analogous Staff. The post is superannuated.

Further information from, and applications (curriculum vitae and names of two referees) to. Professor John Reid, Department of Materia Medica, Stobbill General Hospital, Glasgow, within 7 days.

In reply please quote Ref. No. 4870M. (9970)A

#### THE CHINESE UNIVERSITY OF **HONG KONG**

Applications are invited for the following posts tenable from August, 1982:

 Lecturer in Biology (Botany) —
 Applicants should have special competence in genetics or cytology and be able to teach basic botanical courses

2. Lecturer in Chemistry - To teach Physical Chemistry courses with laboratory supervision and to develop a research programme. (Applicants should have some working knowledge of Chinese, and forward a short research proposal (not over 2 pages) listing equipment and support required).

General Requirements: Applicants should have a higher degree (preferably a PhD) in the relevant field, with appropriate university teaching and/or research experience.

Conditions of Service: Annual salary: HK\$112,980 — HK\$128,220 by 2 increments BAR 135,840 — 189,060 by 7 increments. Exchange rate approximately £1 = HK\$10.5. Starting salary will depend on qualifications and experience. Terms of service include include superannuation (University 15%, appointee 5%), medical benefits, education allowance for children long leave, housing benefits for those whose annual salary is \$120,600 and above and, for those employed on overseas terms, accommodation on campus (at a rental equivalent to 7 ½ % of salary) and air passage as well.

Application Procedure: Further information concerning Terms of Service and Application Forms are obtainable from the Personnel Section, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong. Completed application forms together with one t of major publications and copies of testimonials should be returned before January 31, 1982. Please mark 'Recruitment' on cove

(W508)A

#### YOUNG INVESTIGATORS **MOLECULAR BIOLOGY OF HUMAN HEMOPHILIAC FACTOR**

Immediate opening for three young investigators or postdoctoral fellows to address central issues in the molecular biology, biosynthesis, structure and function of the human hemophiliac factor protein. Candidates must have demonstrated accomplishment in applicable areas protein biochemistry, primary structure analysis, or protein biosynthesis and in vitro translation. Experience in trace protein purification and use of immunochemical methodology is desirable.

Appointments are for two years with reappointment dependent upon accomplishment and progress. Salaries are competitive and appropriate to the level of experience and appointment. Starting dates as soon as possible after Jan 1, 1982. Inquiries accompanied by curriculum vitae to Dr T S Edgington, Research Institute of Scripps Clinic, La Jolla, (NW096)A CA 92037.

#### KING'S COLLEGE HOSPITAL MEDICAL **SCHOOL**

(University of London) Denmark Hill, London SE5 8RX

#### RECENT SCIENCE **GRADUATE**

required as research assistant to join a team studying genetic aspects of immune responses in liver disease. No previous experience is necessary as the successful applicant will be trained in tissue typing techniques. The appointment will be for one year in the first instance. Salary £5,285 + £967 London Weighting Allowance, according to age, experience and qualifications.

Applications, in duplicate, including the names and addresses of 2 referees should be sent to the Secretary of the Medical School, at the above address, no later than 17 (9939)A December 1981.

#### IMPERIAL CANCER RESEARCH FUND TECHNICIAN/RESEARCH **OFFICER**

required to assist in a programme of research involving the development of reagents for the diagnosis and treatment of a variety of human tumours. These studies will involve extensive use of monoclonal antibodies and experience in tissue culture and/or immunology would be an advantage. The appointment is based at our Medical Oncology Unit, St Bartholomew's Hospital, London EC1 and the Institute of Child Health, Great Ormond Street Hospital. HNC/Degree. Salary range £6,045 to £8,423

For further information and Application form write to or telephone Miss S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 on 242 0200 ext 305. Quoting reference 12/82

(9930)A

#### ASSISTANT PROFESSOR **OF ANATOMY**

Applications are invited from neurobiologists who have at least two years of postdoctoral research training and who are willing to acquire a suitable background for teaching gross anatomy to professional students. Research interests in neuroendocrinology, developmental neurobiology or sensory neurophysiology or sensory neurophysiology are particularly welcome. If a candidate's research involves primates, a joint appointment with the Yerkes Regional Primate Research Center can be considered. Deadline for receipt of applications is January 1, 1982.

Send curriculum vitae, names of four referees and desciption of specific research plans to; Dr J Sutin, Chairman, Department of Anatomy, Emory University school Medicine, Atlanta, Georgia 30322 Emory University is an Equal Opportunity/Affirmative Action (NW094)A Employer.

#### **POSITION OPEN IN EGYPT**

**Laboratory Director for the Central Laboratory** Located on Campus of Cairo University in Cairo, Egypt

#### Agricultural Development Systems (ADS): **Egypt Project** University of California

Qualifications:

Candidate must have PhD or equivalent in a branch of Plant Biology or Plant Chemistry; experience in scientific investigation related to biological, chemical, and physical analyses; knowledge of use and maintenance of scientific laboratory equipment; experience with training and supervision of technical personnel; and ability to administer a scientific laboratory.

Responsibilities and Duties:

- Organize and supervise the establishment of the laboratory administrative and research subunits. This includes the recruitment of qualified personnel and the preparation of the physical facilities.
- Prepare existing laboratory and equipment for immediate use Supervise the operation and maintenance of the Central
- Laboratory.
- Establish a training program for ADS scientists and visiting scientists and technicians.
- Implement the research instrumentation requirements of the Central Laboratory and, eventually, satellite laboratories elsewhere in the country.
- Advise, consult, and assist ADS project participants in the planning and execution of the research programs.

Annual Base Salary:
Depends upon qualifications. Salary is supplemented by an overseas 'post differential" (currently 15%). Benefits are in accordance with University and AID regulations. The term of appointment is approximately two years.

Final filing date January 31, 1982, or until filled.

Apply to: David Robinson, Agricultural Development Systems: Egypt Project, University of California, Davis, Ca 95616.

The University of California is an equal opportunity/affirmative action employer.

# BIOTECHNOLOGY

Codon's facility located on the San Francisco peninsula is staffed and fully equipped to undertake applied research in nucleic acid chemistry, molecular biology, microbiology, immunology and protein chemistry.

The proximity to several pioneer institutions in recent advances in genetic engineering make the scientific climate in the area highly desirable.

If you wish to join Codon's expanding staff of highly qualified personnel, please send résumé in confidence to:

> Codon \* 430 Valley Drive Brisbane, CA 94005.

> > (NW109)A

# Biochemist / Pharmacologist

Eli Lilly & Co is one of the world's largest producers of ethical pharmaceuticals, animal health and agro-chemical products. At our U.K. Research Centre, situated near Camberley in Surrey, we are actively engaged in basic research on a variety of projects.

We currently have a vacancy for a Biochemist Pharmacologist to work in our Immunology Group on a project dealing with biochemical aspects of membrane function. We are seeking someone with a graduate level or equivalent qualification and preferably, 2-3 years' experience in lipid biochemistry, membranes or enzymology.

For this position a competitive salary is offered together with a non-contributory pension and life assurance scheme, subsidised BUPA, 21 days' holiday, flexible working hours and relocation expenses where applicable.

Interested candidates should telephone or write to Miss S M Smith, Personnel Manager, by the first week in December and interviews will be carried out early in January.

(9966)A



#### LILLY RESEARCH CENTRE LTD.

SURREY. TEL: (0276) 73631

# **MICROBIOLOGY SECTION HEAD**

Permanent staff Scientist position responsible for both fundamental and applied research direction of a 10 + person antitumor drug Microbiology Section in the NCI-FCRF Fermentation Program. Individual to supervise staff scientists in the areas of Prescreen Development, Novel Drug Dereplication (biosynthesis, mode of action) and Strain Development in support of NCI experimental drug production efforts. Requires PhD in microbiology, biochemistry or genetics with experience in antitumor drugs, molecular genetics, streptomyces genetics and rDNA methodology. Related industrial, scientific and supervisory experienced desired. Send curriculum vitae and names of references to:

Personnel Department, Position 2181T, NCI-Frederick Cancer Research Facility, Litton Bionetics, Inc., PO Box B Frederick, MD 21701 EOE M/F

(NW099)A

#### HILL FARMING RESEARCH ORGANISATION Information Officer/Librarian

Applications are invited for the post of Information Officer/Librarian. Candidates should have a degree or HNC or equivalent in Information Science or Librarianship or equivalent experience and preferably with an additional qualification in Science or Agriculture.

The post will involve various aspects of information and public relations work, editorial duties and responsibility for an efficient information retrieval service and library.

Salary: Within the scale £7,467 to £9,184.

Other Conditions: Non-contributory superannuation scheme; Annual leave - 22 days per annum.

Further particulars and application forms may be obtained from the Secretary, Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY to whom they should be returned not later than 31 December 1981. Please quote reference A/6/264

(9949)A

#### **BROOKLYN BOTANIC GARDEN** RESEARCH PLANT **PHYSIOLOGIST**

Position at the Brooklyn Botanic Research Center, Ossining, New York. Research program involves innovative approaches to basic and applied research mostly with a horticultural bias. Interest and expertise in tissue culture highly desirable. PhD required.

Send, in confidence, curriculum vitae, statement of research interests, salary history, three letters of reference, graduate transcripts and publications to: Dr Stephen K M Tim, Director of Scientific Affairs, 1000 Washington Avenue, Brooklyn, New York 11225. An Affirmative Action/ Equal Opportunity Employer. (NW093)A

#### UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF PHARMACOLOGICAL SCIENCES TEMPORARY CLINICAL/ **NON-CLINICAL** RESEARCH ASSOCIATE

Applications are invited from suitably qualified candidates with experience in neurophysiology for a temporary post of Research Associate to join a team studying somatosensory evoked potentials for the objective measure-ment and differential diagnosis of chronic pain. This project will provide opportunities for experience in a clinical psychopharmacology laboratory and/or a Pain Relief Clinic. This temporary appointment will start as soon as possible and is available fro three years. Com-mencing salary according to age, qualifications and experience up to £10,360 pa on the scale £8,070 — £14,300 pa for medically qualified candidates, or £9,335 pa on the scale £6,070 — £10,575 for non-medical candiates.

Applications giving names of two referees should be submitted as soon as possible and not later than 18th December 1981 to Professor J W Thompson, Clinical Psychopharmacology Unit, 13 Framlington Place, Newcastle upon Tyne NE2 4AB, from whom further information can be (9941)A

#### BIOPHYSICIST

tenure track position will be available in Biophysics at the University of Illinois at Urbana-Champaign, effective Fall 1982. The position is expected to be at the assistant professor level. It is anticipated that the successful applicant will carry out research and teaching in biophysics, in the general area of biomolecular structure. Candidates should have a PhD degree and some postdoctoral experience. Salary is netotiable. Please send your curriculum vitae by February 15, 1982 to Dr Thomas G Ebruary 15, 1982 to Dr Itionias of Ebrey, Chairman of Biophysics Search Committee, 524 Burrill Hall, 407 S Goodwin, University of Illinois, Urbana, IL 61801 (Tel: [(217) 333-2015]. The position is available subject to budgetary approval. An Equal Opportunity and an Affirmative Action Employer an Affirmative Action Employer (NW100)A

**UNIVERSITY** OF BIRMINGHAM Faculty of Medicine and Dentistry DEPARTMENT OF CARDIOVASCULAR MEDICINE **POSTDOCTORAL** RESEARCH FELLOW

Applications are invited for the above position to work on the biochemical role of the contractile and regulatory proteins in the mammalian heart. The successful applicant will join the Molecular Cardiology Unit within the Department investigating the mode of action of myosin isoenzymes in relation to cardiac function. Particular emphasis is placed on the study of the human myocardium and the molecular changes occurring during development and cardiac disease. Experience in protein separation and modification techniques preferable.

The post is available for 2 years and 5 months, supported by the MRC, at a starting salary between £6,070 to £6,880 plus superannuation.

Further information from Dr P Cummins, Cardiovascular Medicine, University of Birmingham B15 2TH (021-472 1311 ext 224).

Applications (three copies) to Assistant Registrar, Medical School, Birmingham B15 2TJ by 14 December 1981. Please quote ref RF/Card/481. (9958)A RF/Card/481.

#### The University of Arizona Seeks a Dean of the Faculty of Science

The University of Arizona is creating a new College of Arts and Sciences to be composed of the Faculties of Fine Arts, Humanities, Sciences, Social and Behavioral Sciences. The Faculty of Sciences will consist of about twelve departments including mathematics, the physical, and biological sciences in a growing series of a major programs on our campus. The administration recognizes the scientific achievements of its faculty and now desires to enhance both the cohesion and leadership in these disciplines by means of the new organization.

The University is seeking candidates for the Dean of Faculty of Science who have the highest qualifications, both in academic and administrative accomplishments. The appointment will also include a professorship with tenure. The Dean will be responsible for academic, administrative and budgetary activities within his faculty. and will report to the Provost of the College of Arts and Sciences. All inquiries, applications, and nominations should be sent to the Office of the Executive Vice President no later than February 1, 1982.

A. B. Weaver, Executive Vice President, University of Arizona, Administration 512, Tucson, Arizona 85721.

Equal Opportunity/Affirmative Action Employer.

(NW108)A

#### THE UNIVERSITY OF **NEBRASKA**

Lincoln

Molecular Genetics

#### ASSISTANT/ASSOCIATE **PROFESSOR**

tenure-leading, August, 1982. Conduct an active research program on molecular aspects of gene organization, gene replication or gene expressing. Teaching res-ponsibilities will include microbial genetics graduate course and molecular biology or microbiology course. \$19,500 minimum salary for academic year. Research initiation funds available.

Send curriculum vitae, summary of research interests and names of three referees by February 1 to: Dr S D Schwartzbach, School of Life Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588-0118. Afirmative Action/ Equal Opportunity Employer (NW098)A

#### **UNIVERSITY OF HONG KONG**

#### **CHAIR OF CHEMISTRY**

Applications are invited for the Chair of Chemistry which will fall vacant in January 1983 following the resig-nation of Professor D S Payne.

Annual salary (superannuable) will be within the professional range and not less than HK\$250,920 (£1 HK\$10.60 approx).

At current rates, salaries tax will not exceed 15% of gross income. Housing benefits at a rental of 7½% of salary, education allowance, leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or from the Appointments Unit, Secretary's Office, University of Hong Kong, Hong Kong.

The closing date for application is 31 December 1981. (9952)A

UNIVERSITY OF GEORGIA DEPARTMENT OF BIOCHEMISTRY, **BIOLUMINESCENCE LABORATORY** 

#### Athens, GA 30602 RESEARCH ASSOCIATE

Immediate opening in a project to study the structure and other properties of proteins involved in the bioluminescence of luminous bacteria (Lumazine protein; J Biol Chem (1980) 255, 8804). Recent PhD in Chemistry or Biochemistry, some experience in protein chemistry or physical biochemistry would be an advantage. Inquiries to Dr John Lee. (NW095)A

#### PUBLIC HEALTH LABORATORY SERVICE **BOARD** PHLS CENTRE FOR APPLIED MICROBILOGY AND RESEARCH **PATHOGENIC MICROBES** RESEARCH LABORATORY **BASIC GRADE**

A graduate in microbiology is required to join a group studying the pathogenesis of Legionnaires' disease in experimental animals. The work will include isolation and cultivation of Legionella pneumophila from tissues.

MICROBIOLOGIST

The appointment will be as Basic Grade Microbiologist in the salary range £5,667 — £6,745.

#### JUNIOR 'B' MLSO

Required to assist with studies on experimental Legionnaires' disease. The salary will be in the range £3,120 - £4,268.

Both these posts are funded by the Medical Research Council and will run for one and half years.

Further details of the posts may be obtained from Dr A Baskerville. Tel: 0980 610391.

Applications including curriculum vitae and the names and addresses of two referees should be sent to Mrs M Bushby, Personnel Officer, PHLS Centre, For Applied Microbiology and Research, Porton Down, Wilts SP4 0JG. Closing date 11th SP4 0JG. Cl December, 1981. (9943)A

- a leader in the commercial application of biotechnology is seeking:
- Ph.D.s in molecular biology with experience and interest in virology and infectious diseases. Preference will be given to individuals with a broad background which includes recombinant DNA techniques and immunological characterization of viral antigens. Positions are available at the Sr. Scientist, Scientist, and Postdoctoral levels.
- •Ph.D.s in biochemistry or molecular biology with 3-5 years' postdoctoral experience, preferably in DNA cloning, expression and eukaryotic gene regulation for pharmaceutical use.

Applicants should send their resumes in confidence to:

> **CETUS CORPORATION BOX NJN** 600 Bancroft ₩ay Berkeley, CA 94710 (415) 549-3300



(NW113)A

#### Manchester Area Health Authority (T)

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE Paterson Laboratories

# **Research Assistant**

required to undertake the running of a research project concerned with electron microscope studies of human chromosomes. The project is in progress at present and involves the SEM karyotyping of human metaphase chromosomes from patients carrying abnormal chromosome constituttions with a view to further use in improved clinical diagnosis.

Parallel studies of chromosome structural organisation are being

Applicants must have experience in the techniques of electron microscopy and be familiar with human karyotype.

The project is financed by an NHS Regional Research Grant and is based in the Section of Ultrastructure, Paterson Laboratories, in collaboration with the Department of Medical Genetics, St. Mary's Hospital, Manchester.

Commencing date 1 January 1982 continuing until 31 May 1984.

Salary scale in the range £5,886 to £7,537 according to age and experience.

Applications in writing with full Curriculum Vitae to Dr. T. D. Allen at the Paterson Laboratories, Christie Hospital, Wilmslow Road.

Manchester M20 9BX.

(9945)A

Manchester South District

#### TWO MOLECULAR **GENETICISTS**

#### Department of Molecular and **Population Genetics** University of Georgia

The University of Georgia is seeking two molecular geneticists for tenure track positions at the Assistant Professor level, beginning fall 1982. For one position, preference will be given to candidates with interests in prokaryotic or viral genetics. For the other position, preference will be given to a eukaryotic molecular geneticist; one area of interest is molecular evolution. However, qualified candidates in any area of molecular genetics will be considered. Candidates will be expected to maintain an active research program and to teach at the graduate and undergraduate level. The Department of Molecular and Population Genetics is a new department with 12 faculty members and strong research programs in molecular genetics and population genetics.

Applicants should send curriculum vitae and names and addresses of four references to Dr. Norman Giles, Chairman, Molecular Geneticist Search Committee, Department of Molecular and Population Genetics, University of Georgia, Athens, Georgia 30602. Screening of applicants will begin immediately. The closing date for applications is February

An Equal Opportunity/Affirmative Action Employer. (NW106)A

#### HIGH ALTITUDE OBSERVATORY

Visitor Appointments at the High Altitude Observatory are available for new and established PhD's for up to one year periods to carry out research in solar physics, solar-terrestrial physics, and related subjects. Applicants should provide a curriculum vitae including education, work experience, publications, the names of three scientists familiar with their work, and a statement of their research plans.

Applications must be received by 15 January 1982, and they should be sent to: Visitor Committee, High Altitude Observatory, National Center for Atmospheric Research (NCAR), PO Box 3000, Boulder, Colorado 80307. NCAR is an Equal Opportunityy/Affirmative Action Employer

(NW931)A

#### YOU'VE GOT A BRIGHT IDEA

FOR NEW BIOLOGICAL OR **CHEMICAL COMPOUNDS** 

WE HAVE THE CAPITAL TO DEVELOP NEW DRUGS

We are not a pharmaceutical firm but a Swiss-based, independant financial group which is prepared to let you participate in a capital venture for the exploitation of your project.

Our object: the development of new biological or chemical products with previous pharmalogical screening up to the stage of clinical tolerance and efficacy required to permit licensing negotiations.

To obtain all the information required for initial selection, please write in English, French or German to:

DEBIOPHARM S.A. Petit-Chêne 38 - 1001 LAUSANNE (Switzerland).

(W368)A

#### **BIOCHIMISTE/PHARMACOLOGUE** — **REGION PARISIENNE**

Un des principaux groupes pharmaceutiques français du secteur privé recherche un biochimiste expérimenté orienté vers la pharmacologie moléculaire pour un poste de haut niveau, avant une expérience d'au moins cinq ans dans l'industrie pharmaceutique, ou ayant dirigé une importante Unité de recherches à l'Université, de préférence dans le domaine cardiovasculaire, SNC ou sécrétion gastrique.

Ce poste conviendrait à un candidat pouvant prouver son expérience par une liste importante de publications.

Envoyer CV et liste des publications sous référence W502, c/o Nature, Macmillans Journals, 4 Little Essex Street, London WC2R 3LF.

Discrétion assurée.

(W502)

#### The Queen's University of Belfast

#### RESEARCH ASSISTANT Marine Biology Station, Portaferry

This NERC financed project tenable for three years will be concerned with a study of factors contributing to increases in phytoplankton productivity and biomass in oceanic shelf-break and frontal zones. An ability to undertake work at sea will be necessary.

Commencing salary £5,286 rising to £6,072 per annum with USS.

Further particulars may be obtained from Dr G Savidge, QUB Marine Biology Station, Portaferry, Co Down BT22 IPF, Northern Ireland. Applications, giving the names and addresses of two referees,

should be sent to the Personnel Officer, The Queen's University Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. Closing date: 14 December 1981 (Please quote Ref 81/N) (9957)A

#### PROFESSIONAL RESEARCH ASSISTANT IN CARBOHYDRATE CHEMISTRY

To participate in the purification and structural analysis of biologically interesting complex carbohydrates using modern instrumentation such as NMR, GC, GC-MS, LC and LC-MS. Applicants should have the equivalent of a BSc or MSc in Chemistry or Biochemistry. Starting salary (between \$12,000 and \$16,000) will depend on experience. The University of Colorado is an affirmative action/equal opportunity employer.

Applications should be sent to Michael McNeil or Peter Alber-sheim, Department of Chemistry, University of Colorado, Campus Box 215, Boulder, Colorado 80309, and should be received by December 31, (NIWOR9)A

#### A PROFESSORSHIP (C2)

will be available in the Department of Medical Microbiology at the Free University of Berlin. Preference will be given to a person with ample research experience in the field of T-cell dependent anti-infectious immunity. Obligations are, besides research, teaching and participation in the diagnostic bacteriological routine work, for the latter activity, no particular experience necessary.

For further information contact: Prof. Dr med. Helmut Hahn, Institute für Medizinische Mikrobiologie der Freien Universität Berlin Hindenburgdamm 27, D-1000 Berlin (W498)A 45. West Germany.

#### **WANT TO LIVE** IN THE U.S.A.? American agency

provides help.

For free information write the U.K. address.

The Transatiantic Agency, (N), 33 Great James Street, London WC1. Enclose SAE please

(9891)A

#### **IMMUNOLOGIST** FRED HUTCHINSON CANCER RESEARCH CENTER

The Fred Hutchinson Cancer Research Centre conducts a broad based program in basic biological sciences. The Center wishes to recruit a faculty scientist with an established record of accomplishment in basic immunology to join this program. Considerable space and resources are available. Interested individuals should submit curriculum vitae and suggested references by December 20, 1981 to:

Associate Director for Scientific Programs, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104.

FHCRC is an Equal Opportunity/Affirmative Action employer (NW103)A



#### MLSO OR BASIC GRADE BIOCHEMIST

There is a vacancy for a Medical Laboratory Scientific Officer or a Basic Grade Biochemist to assist in routine Clinical Biochemistry Laboratory. The work is varied and may provide an opportunity to gain experience in some special techniques for estimating biogenic amine metabolism, for which the laboratory is a reference centre.

Applications with names of referees to Professor M.
Sandler, Queen Charlotte's Maternity Hospital, Goldhawk
Road, London W6. (9934)A

#### UNIVERSITY OF WESTERN ONTARIO Cancer Research Laboratory POSTDOCTORAL FELLOWS

Staff includes Drs. G. Chaconas, K. Ebisuzaki, E. Faust, D. Fujita and B. Milavetz. Areas of research include DNA replication, poly (ADP)ribose metabolism, tumour virology, control of mammalian cell cycle, and cloning of mammalian genes with particular reference to cancer. Submit curriculum vitae and letters of reference to Dr. David Denhardt, Cancer Research Laboratory, University of Western Ontario, London N6A 5B7 Canada. (NW102)A

JUNIOR Laboratory Scientific Officer required in Department of Microbiology for one year to assist in clinical research project on viruses in chronic arthritis. Previous experience in virology or cell culture an advantage. Salary in range £2,929 — £4,055, or £3,120 — £4,220, according to age and qualifications, plus £859 London Weighting. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. MC4. (9967)A

## UNIVERSITY OF OXFORD

DEPARTMENT OF AGRICULTURAL AND FOREST SCIENCES RESEARCH ASSISTANT

Applications are invited for an ARC-funded post, to work on the physiology of disease resistance mechanisms in conifer roots, in collaboration with Dr R B Pearce. This post, which is tenable for three years, will commence on 1st February 1982 or as soon as possible thereafter. Salary range £5,285 to £8,925 pa.

Applicants should have research experience in plant pathology, plant biochemistry, or an allied field.

Applications, including a curriculum vitae and the names of two referees should be sent by 18th December 1981 to the Administrator, Department of Agricultural and Forest Sciences, Commonwealth Forestry Institute, South Parks Road, Oxford OX1 3RB from whom further particulars may be obtained. (9954)A

# CHEMISTRY University of Toronto SCARBOROUGH COLLEGE

A one year limited-term Assistant Professor appointment in Organic Chemistry or Physical Chemistry available July 1 or September 1, 1982. Doctorate required with expertise in Organic Chemistry or Physical Chemistry. Duties include undergraduate teaching and research.

Send curriculum vitae and names of three referees to: John E. Dove, Chairman, Physical Sciences Division, Scarborough College, 1265 Military Trail, West Hill, Ontario, Canada M1C 1A4. (NW115)A

# IMMUNOLOGY OF DIABETES

Applications are invited for the position of assistant or associate professor to work in the area of the aetiology of diabetes. The position, available on July 1, 1982, will carry an initial appointment of five years funded through *The Banting and Best Diabetes Centre, University of Toronto* at a competitive salary

Candidates should have a strong background in immunology and be interested in developing a strong research program in the areas of autoimmunity and/or immunogenetics related to diabetes. A colony of partially inbred (11 generations) spontaneously diabetic, BB Wistar Rats is available for research purposes.

The successful candidate will be expected to attract independent research grants and to spend at least 75% of his/her time carrying out an independent research program. Teaching obligations will be minimal.

Applicants should forward a curriculum vitae, an outline of Research Interests and the names of three references to Dr David H Maclennan, Professor and Chairman, Banting and Best Department of Medical Research, 112 College Street, University of Toronto, M5G 1L6, Toronto, Ontario. (NW112)A

#### QUEEN'S UNIVERSITY Kingston, Ontario

The Department of Biology invites applications for three positions. Successful applicants will be expected to teach in the undergraduate program and develop vigorous research and graduate education programs. Appointments will be effective September 1, 1982 and are expected to be at, but are not restricted to, the rank of Assistant Professor with salary commensurate with qualifications (floor — \$20,800). Equal opportunities exist for qualified male or female applicants. First consideration will be given to Canadian citizens or landed immigrants. All positions require a PhD degree and published evidence of excellent research ability.

#### **PLANT ECOLOGIST**

Tenure track position. Preference will be given to persons with active interests in population or community ecology of land plants. Research program will be expected to emphasize a quantitative experimental approach and the application of ecological and evolutionary theory to problems of plantecology. It is expected that the successful applicant will interact closely with the present faculty in population biology.

#### PLANT DEVELOPMENT

Tenure track position. Applicants must have expertise in plant cell and tissue culture, including plant regeneration and are expected to be able to interact closely with present faculty in plant physiology/development and recombinant DNA technology.

#### **CELL BIOLOGIST**

One year term appointment with the possibility of renewal. Applicants are expected to have research interests in animal cell biology, possibly including fine structure, and be able to interact closely with present faculty in cell biology. Successful applicant must be able to teach in a first-year cell structure and function course.

Application deadline is April 1, 1982 or until a suitable candidate is selected. Send applications, which should include a curriculum vitae, statement of future research interests and names and addresses of three referees, to Professor D. T. Canvin, Head, Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

(NW104)A

Agricultural Scientific Services, East Craigs, Edinburgh

Department of Agriculture and Fisheries for Scotland

# **Plant Pathologist**

. . . to lead a team assessing crop losses caused by diseases and pests (and possibly weed competition) and to investigate the cost/benefit of pesticide application to prevent such losses. The cost/benefit studies are expected to include a consideration of the effects of pesticide use on wildlife and the environment generally as well as the straightforward assessment of economic gains or losses.

Candidates, normally aged under 32, should have a good honours degree in a relevant discipline and at least 4 years' experience in agricultural plant pathology.

Appointment as Senior Scientific Officer £8,205 — £10,320 with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 18 December 1981) write to Scottish Office, Personnel Division, E-PM(PTS)3, Room 208, 16 Waterloo Place, Edinburgh EH1 3DN, telephone 031-556-8400 extension 5088 or 4311. Please guote ref: SA/20/DLA.

(9959)A



#### **EMBO PRACTICAL COURSE**

#### Automated Chemical and Enzymic **Gene Synthesis**

Department of Biochemistry Technische Hochschule Darmstadt

#### MARCH 21st TO APRIL 3rd, 1982

The teaching staff will include:

J.H. van Boom (Leiden), M.H. Caruthers (Colorado), H.J. Fritz (Köln), M.J. Gait (Cambridge, UK), H.G. Gassen (Darmstadt), W. Hillen (Darmstadt), K. Itakura (City of Hope, LA), H. Kossel (Freiburg), H. Köster (Hamburg), K.E. Norris (Bagsvaerd), E. Ohtsuka (Osaka), H. Schott (Tübingen), H. Selinger (Ulm), O.C. Uhlenbeck (Urbana), E.L. Winnacker (München) and others.

The programme will consist of practical work, lectures and seminars on solid-phase chemical synthesis of oligodeoxynucleotides

- phosphotriester method as related to the phosphoroamidite procedure
- automated oligonucleotide synthesis
- oligonucleotides from DNA fragmentation enzymic ligation of oligonucleotides
- recombination of RNA
- survey of oligonucleotide separation and analysis
- ligation of synthetic genes (promotors) to plasmid vectors

A maximum of 15 students will be accepted. Applications should include a short curriculum vitae and a description of present research interets. The registration fee for the practical course is 200, DM, which does not include food and lodging. A small number of grants in aid to cover partially board and lodging expenses may be granted to participants who are unable to obtain funds from other sources.

The weekend MARCH 27/28th will be kept free from practical work to allow the participation in a WORKSHOP entitled

#### **Prospects of Automation in Gene Synthesis**

Informal application is sufficient for the workshop. The number of participants will be limited to 100. The registration fee is 60, DM.

#### THE CLOSING DATE FOR APPLICATIONS IS JANUARY 15th.

Those accepted will be notified not later than January 31st, 1982. Applications should be sent to Dr H.G. Gassen, T H Darmstadt, Institut für Organische Chemie und Biochemie, Petersenstrasse 22, D-6100 Darmstadt. Phone: 06151/163657, Telex: THD 419579 (W504) C

#### SEMINARS and SYMPOSIA

#### George Washington **University Spring** Symposium on Health Sciences (Washington, D.C. — May 18-21, 1982) Gene Expression '82

The conference will feature five sessions on gene structure, expression and gene transfer. Chromatin Structure: Gary Felsenfeld (NIH), A. Varshavsky (MIT), S. Elgin (St. Louis); Gene Structure: P. Leder (NIH), M. Wormington (Carnegie), Gene Structure: P. Leder (NIH), M. Wormington (Carnegie), A. Efstratiadis (Harvard), L. Kedes (Stanford), J. Seidman (NIH); Gene Expression: D. Hamer (NIH), G. Khoury (NIH), M. Wigler (CSH); Cell Free Systems: R. Roeder (St. Louis), P. Sharp (MIT), C. Parker (Caltech), B. Solnar-Webb (Johns Hopkins); Gene Transfer: F. Anderson (NIH), F. Ruddle (Yale), W. Salser (UCLA) and P. Hoppe (Maine).

There will be open registration for a limited number of participants including those who wish to make poster presentations. For further information write to:

australia de la constantia de la constanti

A. Kumar, Dept. of Biochemistry, George Washington University, School of Medicine, 2300 Eye St., N.W.
Washington, D.C. 20037. (202) 676-4415. (NW105)M

#### STUDENTSHIPS

#### ST JOHN'S COLLEGE, Cambridge **RESEARCH STUDENTSHIPS**

The College now invites applications for a number of Research Studentships from men and women who are not already members of the College but who propose to register as PhD students at the University. The Studentships will normally be tenable for three years and of such value as will, in addition to the payment of fees, bring the student's total emoluments to £2,345 a year. Candidates should have gained or have a strong prospect of gaining a first-class honours degree or equivalent.

Further details and application forms can be obtained from the Senior Tutor, St John's College, Cambridge CB2 1TP. Completed applications must be received by 1 April 1982. (9956)F

#### A CALL FOR PAPERS

#### FOURTH INTERNATIONAL CONFERENCE ON PHYSICO-CHEMICAL HYDRODYNAMICS

June 14 through 17, 1982

#### The Roosevelt Hotel Madison Avenue and Forty-Fifth Street **New York City**

The Fourth International Conference on physico-chemical hydrodynamics organized by the New York Academy of Sciences and the City College of the City University of New York will concentrate on the following: Interface mechanics and multiphase systems; theory of turbulence and its applications; transport phenomena, chemical reacting systems and low reynolds number hydrodynamics; physico-chemical hydrodynamics of polymers and bio-systems; and physicochemical hydrodynamics in electrochemistry.

Prominent scientists from various countries will present the results of their recent investigations. Everyone interested in presenting a paper is invited to submit a title and a short abstract by February 15 and an extended abstract of two pages, which will be published in the Academy's annual series, by May 1 to one of the conference organizers: Benjamin Levich, Albert Einstein Professor of Science, or Robert Pfeffer, Chairman, both of the Department of Chemical Engineering at the City College of New York, New York, NY 10031.

(NW116)C

#### **ASSISTANTSHIPS**

#### UNIVERSITY OF ST ANDREWS

DEPARTMENT OF PHYSIOLOGY & PHARMACOLOGY

Applications are invited for a

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(9953)P

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For further information, please write or phone: The Course Secretary, W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, NY 12946. 518/523-2427.

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Application forms and further details may be obtained from Dr J Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.

(W510)E

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For further information contact Dr M Fried (Tel. 01-242 0200 ext 297). Applications with curriculum vitae and the names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 England quoting reference no. 9/82. (9932)E

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Further information concerning the fellowships and stipends as well as application forms may be obtained from Dr. John Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 6900 Heidelberg, Federal Republic of Germany.

Completed applications recieved before February 19, 1982 will be reviewed by the Fund Committee on April 30, 1982 and any awards made can be activated immediately.

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DEPARTMENT OF MICROBIOLOGY

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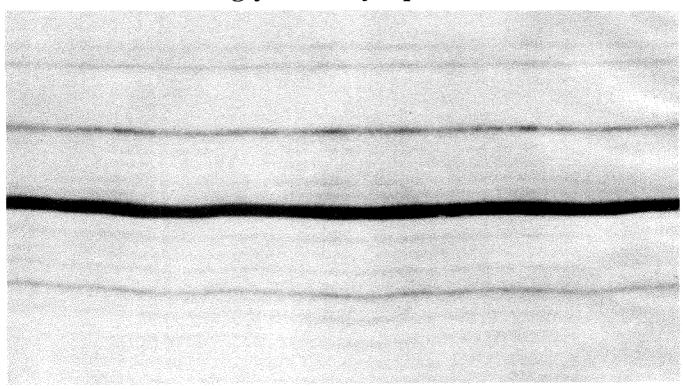
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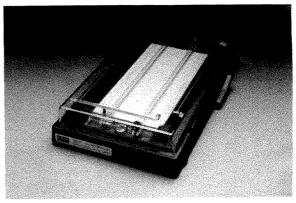
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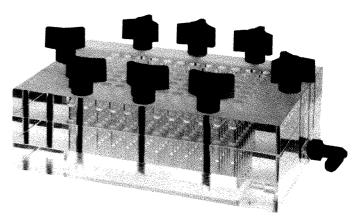


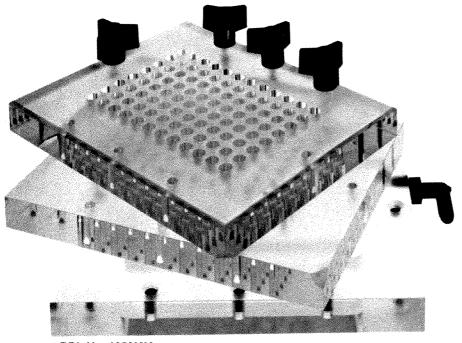
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Normal faults forming part of a long rift-like system extending some 600 kilometres in a north-south direction from Mount Chomolari to the heart of Tibet — one of many similar "rifts" recognized on Landsat photos. Tiny black dots in the meadows (right) are yaks and give the scale. See pages 403, 405, 410 and 414. (Photograph by Kevin Kling.) The Christmas Books Supplement begins on page 483.

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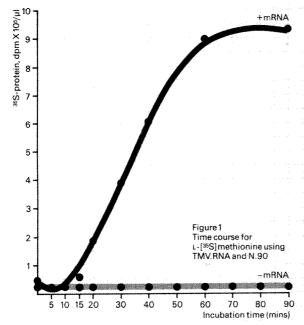
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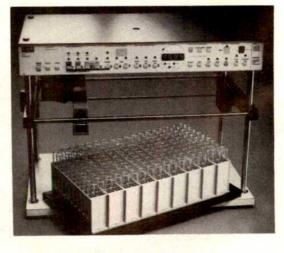
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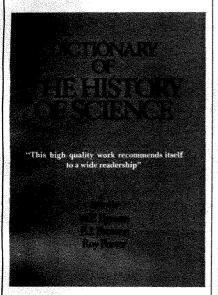


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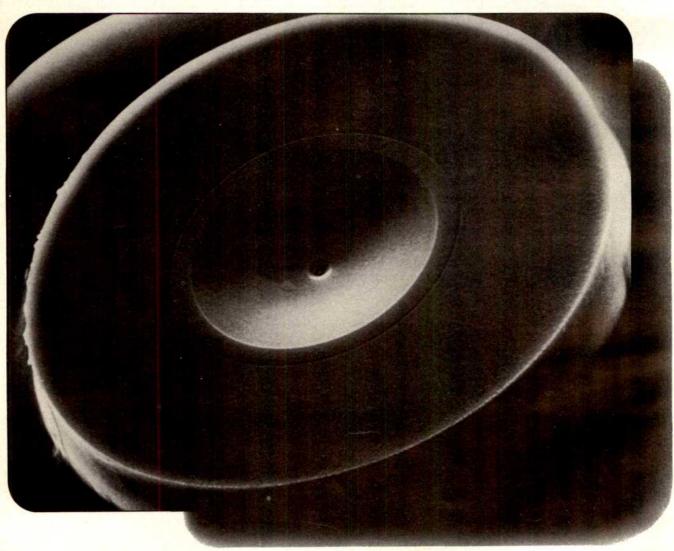
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An enlargement of the etched surface of an optical graded index fibre (50µ core and 125µ cladding diameter).

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very closely resembles the ideal parabola.

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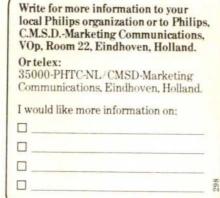


Lightwave telephone transmission. In addition to Philips all digital PRX/D exchanges, Saudi Arabia has ordered 140M/bits optical fibre systems to be integrated into the existing 2M/bits PCM networks in the Jeddah and Riyadh multi-exchange areas. Six-fibre cables, with a capacity of 1920 calls per fibre, will be used in the repeaterless routes, which total some 45 system kilometers. When completed it will be one of the first operational 140M/bits optical fibre transmission systems in the world. Circle No.12 on Reader Enquiry Card.



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## nature

#### 3 December 1981

# Tolerance but no quarter for creationism

Creation-science goes on trial next week in Arkansas. The issue is whether recent legislation establishes religion. It would be better that it should have been the freedom of teachers to say what they believe.

The trial which opens in Arkansas next Monday on the state's decision to legislate for equal time in the school curriculum for creationism and Darwinian evolution will not be as dramatic an occasion as the trial of the Tennessee biology teacher, Mr John Scopes, in 1928. Then the issue was whether a teacher could be prevented by the law from telling his students what Darwin said. (Technically, Scopes was found guilty and fined \$100, but his lawyer Clarence Darrow made such a monkey of the prosecution that the conviction was quashed by the United States Supreme Court.) Now the argument is about a much narrower constitutional issue. By requiring equal time for what is called "creation-science" with Darwinism, is the state of Arkansas violating the provision of the United States constitution that ensures the separation of religion and the state? The American Council for Civil Liberties, which is to its credit bringing suit against Arkansas, has chosen to argue that what is called creationscience, itself the creation of predominantly religious people, cannot be made a compulsory part of the curriculum in state schools without institutionalizing religion.

The issue is far from clear cut. Would it necessarily be a constitutional offence if a state board of education required schools to teach the early history of the Christian Church, plainly a matter of great cultural importance? Part of the weakness of the state's case is that under the "Balanced treatment for creation-science and evolution-science Act", rushed through the state legislature in March this year, the doctrine of equal time is vested in the law, and is not an administrative decision of the board of education. But the essential flaw, likely to fall outside the terms of reference of next week's trial, is the sheer folly implied by the term "creation-science".

Three separate questions arise — the status of Darwinism, the status of "creation-science" and the question of how the components of any school curriculum should be decided. The argument next week will be dominated by attempts to demonstrate that Darwinism and "creation-science" are equally valid explanations of the origin of the natural world. The state attorney-general's decision to call as a witness Professor Chandra Wickramasinghe, Sir Fred Hoyle's collaborator in the theory of life from outer space, is plainly meant to show that even among non-creationist scientists, Darwinism does not have the field to itself. The misfortune in the framework of next week's trial is that there will be no opportunity to challenge the assumption, not only in Arkansas, that state governments can sensibly intervene on specific matters of educational policy.

The status as a scientific theory of Darwinism was predictably well defined by Sir Andrew Huxley in his anniversary address to the Royal Society this week (see page 395). The supposed conflict on which the creationists have seized between the supposed gradualism of Darwinism and the observation that evolution may be episodic (chiefly due to S.J. Gould and N. Eldridge) is not a conflict at all. Gould will be saying just this at next week's trial. Neither is the problem of the "missing links" a problem. Darwinism has all the attributes of a mature and powerful scientific theory — newly discovered and apparently contradictory phenomena such as Mendelian inheritance have turned out to be confirmatory. And with the passage of time, the once perplexing question of how living things came to be the way they are has been particularized to a group of more tangible (if as yet

unanswered) questions, the regulation of embryogenesis and the evolutionary role of consciousness for example.

During the long correspondence on the role of the British Museum (Natural History) in the past year, much has been made of Sir Karl Popper's confusing remark that the theory of evolution is not falsifiable and must herefore be called "metaphysical". Like cladism in its own field, that is a useful way of classifying scientific theories, suggesting what kinds of tests may be considered compelling. But none of this is relevant to the function of the theory of evolution in scientific enquiry. What matters there is that the theory of evolution is found empirically - by biochemists as well as simple biologists - by planning investigations and designing experiments. To pretend that the theory carries no compelling conviction while problems remain unsolved is as ridiculous as it would be to hold that Newtonian mechanics (within its accepted field of validity) will remain unproven so long as the general three-body problem has not been solved.

Creationism, even when dignified as "creation-science", has a quite different status. Many working scientists, finding that it does not help in ordering thought or designing experiments, are tempted to dismiss it as rubbish. Individually, they are right to do so. Collectively, however, the scientific profession would be mistaken to seek to outlaw creationism. Many religious people find creationism a useful way of ordering their own non-scientific thoughts. Some religious Darwinists are also creationists, suggesting that the alternative ways of accounting for the natural world are orthogonal ways of ordering phenomena. But the scientific community's justifiable complaint against the resurgence of creationism in the past few years is that it is now called creation-science. For what will be given equal time in the Arkansas curriculum is not science at all in the sense of being a system for relating all phenomena in a certain domain to a single set of causes which are themselves susceptible to investigation. Toleration of this shoddy pretence has already gone too far, and in surprising places. (Nature's complaint, earlier this year, about evidence of creationist backsliding at the British Museum, is not sufficiently answered by Sir Andrew Huxley's assertion, earlier this week, that the signs were "so slight that most visitors do not even notice them".). The plain truth, which museum exhibitors and state legislators must recognize, is that creationism is not a part of science but an alternative to it.

However the trial next week is decided, the question will remain unresolved of how the curriculum in state schools should be decided. Improbably, the creationists may lose their case, but the right of state governments to specify the content of the school curriculum will be unchallenged. On paper, that right has a simple derivation. State schools are dependants of the state government, and those who teach in them are employees of their local governments. On the principle that public employees must be accountable to their paymasters for all aspects of their work, it is possible to argue that a state is well within its rights to require that teachers should teach whatever is required of them. So would a tobacco-growing state such as Virginia or North Carolina be allowed to instruct its teachers to play down the evidence for a link between smoking and lung cancer? States' rights apart, such a decision, however constitutional, would provoke an outcry that could not be gainsaid. And the American Council for Civil

Liberties would then be filing suit on the grounds of the First Amendment to the Constitution, that which assures free speech—in this case the teachers'.

Exactly the same argument applies to the disastrous legislation that the state of Arkansas has hung around its neck. (In the past few weeks, Mississippi has followed suit.) By requiring that biology teachers should in future teach "creation-science", it is requiring that honest people should tell what they consider to be lies in public. By doing so, Arkansas will undermine the professional integrity of a substantial part of the state's teaching staff and ultimately of the education system as a whole. Is it too much to hope that, when the trial is over — and however it is decided — Arkansas will have the wit to find some way of delegating the fine control of the school curriculum to those on whom the responsibility properly falls — the educationists employed for that purpose?

# What systems analysis?

Laxenburg has a new director but an uncertain future. It also needs a policy.

Not so long ago, in the early 1970s, governments throughout the world were being asked to subscribe to what was intended as a unique instrument of scientific collaboration between East and West — the creation of the International Institute of Applied Systems Analysis at Laxenburg near Vienna. How splendid, the prospectus read, that there should be a research institute (supported principally by the governments of the Soviet Union and the United States) dedicated to the application of objective methods of analysis to problems of contemporary importance throughout the world. Eventually, the misgivings of those who held that the prospectus put the cart before the horse — the objective of East-West collaboration before the definition of a tangible programme of research — were overcome. The institute came into being and has indeed been a place where people, mostly modellers, desk scientists and even social scientists from both sides of the European boundary, have worked alongside each other. The most conspicuous product of this effort has been the study on energy published earlier this year. Now, for two reasons, the institute is in for a sea-change.

The most immediate difficulty is financial. President Reagan's first budget in March deliberately required that the National Science Foundation should reduce spending on overseas activities in general. Nobody appears at the time to have appreciated that United States government support for the Vienna institute is laundered through the National Academy of Sciences until the academy pointed out that it would be unable to pay the subscription due this month. The United States Administration has now found the funds with which the academy can make the contribution legally required of it. Whether it will be able to remain a member in 1983 remains uncertain - and will not be known until the budget for 1983 is published next February. It is, however, a fair guess that if the United States if forced to withdraw, the Soviet Union (whose financial contribution is identical) will also do so. The Laxenburg institute, already somewhat shrunken, must live with uncertainty until the summer.

At first sight, this may seem the worst time for a new director to take charge at Laxenburg. That, however, is the opposite of the truth. If change of some kind is unavoidable, a new director may be an advantage. This is the spirit in which to regard the appointment of Professor C.S. Holling, an ecologist from the University of British Columbia, to Laxenburg with effect from 1 December. he may have to share some of the personal anxieties about the future that afflict the staff of the institute, but he is likely to be less strictly bound by past promises than his predecessors would have been. With a little luck, he may be able to devise a programme of research that can be tailored as the months go by to suit whatever budget becomes available in the year ahead.

But that kind of programme should that be? The trouble so far at Laxenburg is that the original scepticism about the institute has not been stilled by its achievements. The institute has laboured for

a decade and produced not so much a mouse as an elephant — a turgid account of familiar problems in the supply of and demand for energy that might have been illuminating if it could have been published several years earlier. Plainly that is a model for Dr Holling to avoid.

The question remains, however, of what is to be understood by the term "systems analysis" in the institute's title. Computer engineers have a simple answer, but the Laxenburg institute is intended as more than a computing centre. Originally, the phrase may have provided a seemingly innocuous unbrella beneath which people from different economic systems could work together. Now it may be necessary to acknowledge what has always been the truth — that the most useful problems for the institute to tackle are problems that impinge on economics and thus, because economics is the theory of social choice, on politics. What mechanisms should there be, for example, for sharing technological activity among industrialized states, the contemporary equivalent of Adam Smith's classical problem? How most efficiently can the supplies of natural gas to the industrialized world be used? And what can be said about the pattern of manufacturing industry and of world trade in the years ahead? With its unique constitution, the institute is well placed to capture international attention with attempts to answer such unspoken questions. The danger is that it will settle for yet another easy option, and devote itself to important but distant questions such as the problem of the accumulation of carbon dioxide in the atmosphere.

#### Yellow rain too soon

The United Nations commission on poisonous yellow rain in South-East Asia issued an inconclusive report. It would have been wiser not to comment.

The United Nations has done itself a power of harm by its appointment of a commission to investigate charges by the United States that the Soviet Union has been using biological weapons in South-East Asia and in Afghanistan. Last week, the commission (having been to South-East Asia but not to Afghanistan) said that it had been unable to reach a definite conclusion. This week, the commission (which would no doubt prefer to be released from its responsibilities) is likely to be asked by a formal resolution of the General Assembly to soldier on. Dr Kurt Waldheim, the Secretary-General of the United Nations, is no doubt hoping that by the time the next report on the subject appears, his own continued tenure of his important post will have been confirmed.

The tale of the yellow rain over South-East Asia is far too tortuous for anybody's comfort. The allegation that aerial sprays containing unusual amounts of mycotoxins have recently been sprayed over South-East Asia was first raised at the end of last year but was brought more firmly to public attention some weeks ago by the United States Secretary of State, Mr Alexander Haig, in a speech in Berlin. The subsequent tour of European capitals by a group of Mr Haig's choosing was notable partly for the anonymity of its members but chiefly for the vagueness of the extra evidence it was able to produce (see *Nature* 22 October, p.598). The group left those who heard what it had to say with the firm impression that it would have been happier if there had been more substance in what it said.

Unhappily, the passage of time is death for innuendo. Mr Haig charged that the Soviet Union sprayed mycotoxins from the genus Fusarium on innocent people. In was never self-evident why such a stratagem should be followed, even by supposedly malevolent people. Why not anthrax, for example? The classical disadvantage of biological weapons is, after all, well-known to be their slowness. Furthermore, even though further samples of soil containing mycotoxins have been produced, they may be there naturally, and it is premature to claim it as proof that these chemical agents were used. What the US government should do is to publish the data it has to hand in the scientific literature and let others judge for themselves what should be made of it. Jumping the gun is no way to world peace.

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# Fusion chief resigns over budget cuts

# Mirror devices and tokamaks in conflict

Washington

The head of fusion research programmes in the United States Department of Energy (DoE) has resigned in protest at the way in which the Reagan Administration is proposing to allocate research funds between competing magnetic fusion projects.

His decision has apparently been provoked by the decision of the Office of Management and Budget (OMB), in drawing up the recommendation that President Reagan will present to Congress next January, to shift \$25 million from research into mirror devices being studied at the Lawrence Livermore National Laboratory in California to the Tokamak Fusion Test Reactor (TFTR) at the Princeton Plasma Physics Laboratory in New Jersey.

The most immediate impact, according to Dr Edwin Kintner, who has been head of DoE's research programme since 1977, and who last week submitted his resignation as associate director of the Office of Energy Research, will be to delay by about three years completion of the expanded Mirror Fusion Test Facility (known as MFTF-B) which many consider to be the principal competitor to the tokamak.

But the Administration's decision has broader implications, for it effectively undermines a strategy which has been developed by the department under Dr Kintner to diversify magnetic fusion research away from too great a dependence on the success of the Princeton work.

Support for such diversification was first endorsed by the Administration after a report prepared for DoE by an ad hoc committee under the chairmanship of Dr John S. Foster of TRW Inc. in 1978. This pointed out that although the Princeton tokamak programme was achieving impressive scientific results, many engineering problems remained before a viable power reactor could be built.

Concentrating purely on the tokamak, it said, constituted an "unnecessarily high risk". It suggested that the United States should "pursue fusion on a broad front", primarily into mirror devices but also into alternative designs.

After careful review, DoE selected two of these designs for increased support. One was the reversed field pinch, a combination of the tokamak and mirror designs. The other was the Elmo Bumpy Torus (EBT), under development at the Oak Ridge National Laboratory in Tennessee (see

Nature 1 May, p.3).

Mirror research was given further support by the successful development at the Lawrence Livermore Laboratory of tandem mirrors to plug the ends of a solenoid-containing plasma. As a result, it was decided last year to expand the first MFTF into a tandem mirror machine, MFTF-B, scheduled for completion in about four years' time and to perform at temperatures and confinement times comparable to the performance of the tokamak.

This strategy is now coming apart under the stringent budgetary pressures being applied by the Reagan Administration. The first potential casualty is likely to be the EBT "proof-of-principle" machine (EBT-P), proposed for construction by the Carter Administration under contract with McDonnell Douglas, but cut from DoE's budget by Mr Reagan in March. Although Congress has reinstated the money in an appropriations bill now sitting on the President's desk, which would provide \$14 million for EBT-P in the current year, it seems unlikely that he will sign it.

Overall, the 1982 budget for magnetic fusion does not look too bad. Although this was reduced in March from the \$506 million proposed by President Carter to \$456 million — partly by eliminating funds for a centre for magnetic fusion engineering which DoE had previously agreed to build under congressional pressure to move faster on magnetic fusion in general — the latter figure is still

sufficient to maintain a cost-of-living increase for the current financial year.

As with other federal agencies, however, it is the 1983 budget that is giving the greatest cause for concern in Washington as the Administration seeks further substantial cuts in public spending to reduce the federal deficit while increasing the military budget.

OMB's decision to shift support from the mirror programme to the TFTR "is a precedent-setting decision taken without an adequate hearing or technical discussion", Dr Kintner said last week, adding that he had been unable to persuade DoE to appeal and that it was going "to change the whole policy direction of magnetic fusion research for the future".

"For the first time we had a policy which made sense for fusion. It did not argue that we should start competing with other forms of nuclear energy by a definite date, but said we should first meet the short-term objective of knowing whether fusion is practical as an energy source. I worked for five years to create cooperative relationships and research strategies which made sense. That is all dead."

While the strategy of diversified research support has been pursued within DoE, however, it has come under some criticism from the nuclear industry and its congressional supporters who would like to see work proceed on an experimental tokamak reactor as quickly as possible and feel that the DoE strategy may have been overly cautious.

David Dickson

#### Cline stripped of research grants

Washington

In a move clearly intended to warn other scientists not to ignore restrictions laid down by the federal government, Dr Martin Cline of the University of California in Los Angeles (UCLA) has been stripped of one of four research grants he receives from the National Institutes of Health (NIH) because he carried out unauthorized experiments on human patients that involved the use of recombinant DNA techniques.

The incident first came to light last autumn, when it was revealed that, while treating patients in Israel and Italy suffering from  $\beta$ -thalassaemia, Dr Cline used bone marrow cells whose genetic material had been altered by recombinant DNA techniques, even though he had previously told both his university and the two hospitals involved that he would not do so.

Earlier this year, an investigatory committee established by NIH suggested various conditions that should be applied to future grants applications from Dr Cline, who had already resigned as chief of the division of haematology/oncology at UCLA, while retaining his university post as professor of oncology.

In the light of their conclusion that he had violated both federal regulations for the protection of human subjects and NIH guidelines for use of recombinant DNA technology, the members of the committee recommended — and the NIH director (then Dr Donald Fredrickson) accepted — that special prior approval be required for all future grant applications from him for research with either human subjects or recombinant DNA (*Nature* 4 June, p.369).

It was left to the advisory councils of various NIH institutes funding his research to decide what to do about existing grants. Two of them, the National Cancer Institute and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, have taken no direct action.

The advisory council to the National Heart, Lung and Blood Institute (NHLBI), however, decided on a harsher penalty than merely impressing conditions on future applications. It recommended that Dr Cline's current grant from the institute be terminated on 31 March 1982, the end of its first year of support.

The grant, a total of \$244,000, was to have been spread over a period of three years, and covered research into the effec-

tiveness of the insertion of human  $\beta$ -globin genes into mouse haematopoietic cells. A condition of this grant was that no work on human subjects should be involved.

In the case of the National Cancer Institute, two grants for which Dr Cline was the principal investigator were reviewed. The first, for research into normal and malignant haematopoietic cell replication, has been allowed to run until its expiry date at the end of next May, and a renewal application is now under consideration.

On the second grant, part of a four-year project in medical oncology with a total cost of \$3.3 million, the National Cancer Advisory Board has suggested that the grant be continued until 28 February 1982. A renewal grant application has already been submitted by UCLA, with Dr David Golde named to replace Dr Cline as the principal investigator.

Following the decision by Dr Tom Malone (acting director of NIH) to accept the recommendations of the three advisory boards, Dr Cline has pointed out that the termination of the NHLBI grant eliminates only about 20 per cent of his research support. He has also said that he has not decided whether or not to appeal against the decision. In a letter to NIH written in September, however, Dr Cline strongly criticizes the 14-month delay by the UCLA Human Subject Protection Committee in giving a definitive response to his request for permission to carry out experiments.

One sanction recommended by the NHLBI advisory council which was not endorsed by NIH was that Dr Cline be asked to provide assurances that he will not engage in human experimentation involving recombinant DNA for a three-year period. A memorandum from NIH associate director of extramural research, Dr William Raub, says that he does not believe it appropriate for NIH to impose such a sanction, "nor do I believe we have authority to do so". David Dickson

#### ARC biotechnology

#### **Cottage industry?**

The pips may squeak in British research, but biotechnology forges on. The UK Agricultural Research Council (ARC), whose budget shrank 3 per cent in real terms between 1979–80 and 1980–81, has actually been able to announce the setting up of a new research centre. Admittedly, it is in a portable home (a Portakabin), but it will house twelve researchers and provide a focus for ARC work on monoclonal antibodies.

Costing around £100,000 for the Portakabin and equipment, and £100,000 a year to run when it gets going in April next year, the centre will have two resident researchers, four technical staff, and — on average — six visitors from other ARC laboratories.

The goal is to produce a centre of expertise in the handling and creation of

hybridomas — which some ARC virologists have found to be tricky things to culture. The resident researchers at the new centre will work on their own projects (for example, suggest ARC officials, on creating hybridomas of cow, pig and sheep cell lines) and assist visitors with their own problems.

The first applications will be to the creation of specific antibodies to viral strains, such as the varieties of calf enteritis virus, to help research and — perhaps — create vaccines where these would be commercially useful. To this end the unit will also have production facilities large enough to conduct commercial trials.

Who will profit from this ARC commercialism, however, is a moot point. By law the council must pass patent rights to the National Research Development Corporation, now part of the British Technology Group which includes the National Enterprise Board. Unlike the Medical Research Council, ARC has no direct agreement with Celltech, the company created by the National Enterprise Board a year ago to exploit biotechnical developments in British research establishments and universities (and to pursue its own research); but, says Celltech, it would expect the new British Technology Group to consider them as potential developers of any ARC product. The British Technology Group, however, would be free to approach any company it wished — and that might be a new British Technology Group company specializing in agriculture.

ARC recently suggested that the British Technology Group should set up a kind of agricultural parallel to Celltech — in which, no doubt, the ARC would like to have the same exclusive rights and potential earnings as the Medical Research Council has in Celltech — and this idea is still being considered. The British Technology Group will not reveal what stage negotiations have reached, but the ARC do not seem particularly optimistic.

Celltech itself is certainly interested in veterinary applications of hybridomas, but the company does not want to be thought of as specializing only in monoclonal antibodies. Its first and so far only product is a monoclonal antibody against interferon, but Celltech's research and development is now evenly divided between monoclonals and recombinant DNA. Celltech also sees no immediate likelihood of involving itself in the genetic manipulation of plants, unlike the ARC, which has the area very much in mind.

Meanwhile ARC was last week still awaiting official confirmation of the European Council of Ministers agreement three weeks ago to go ahead with the European Commission's biotechnology research programme, which will specialize in agricultural applications. ARC has been closely involved with the definition of the programme, and might hope to win up to £100,000 a year in grants to supplement its

own £1 million annual spending in biotechnology — but only if it has the chance to appoint a strong scientific team to the Advisory Committee on Programme Management. This Brussels committee will ultimately examine research proposals within the programme and — by advising the Commission — effectively hand out the cash. Unfortunately ARC is at a far remove from the Department of Industry, which is in touch with Brussels on this matter, and there are fears that the council may not be approached in time for Britain to get strong representation on the committee.

Robert Walgate

#### Soviet universities

#### Research needed

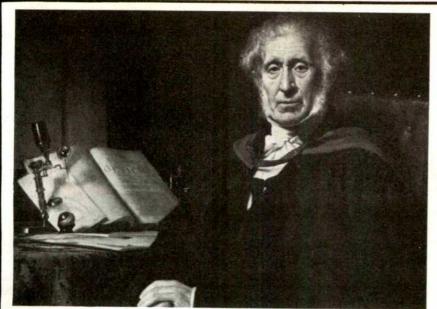
The Soviet Union must invest more in university science, according to Academician Ivan F. Obraztsov, Minister of Higher and Secondary Specialized Education of the Russian Republic. Writing in *Pravda*, he claimed that unless academic research is given priority, it will be difficult to train "good cadres" in the new directions needed for the Soviet economy to develop.

Obraztsov said that Soviet universities and higher educational institutions at present receive no capital funds specifically earmarked for science, although they carry out research worth more than 1,200 million roubles each year. Funds for scientific equipment and research materials are not forthcoming, and computers and similar sophisticated equipment appear far later in the universities than in institutes and laboratories run by specialized ministries.

The lack of funds seems to be especially serious in the engineering faculties. Although Soviet industry is committed to a policy of extensive automation, which is made more urgent by the Soviet Union's falling birth rate, the training of future engineers needs to undergo a "major restructuring". Obraztsov implied that means of familiarizing students with automated design systems, control systems, production lines and manmachine complexes simply do not exist.

The emphasis on engineers reflects a trend in the Soviet press. After a long "press debate" last year on why Soviet public opinion gives more prestige to the pure than to the applied sciences, the weekly *Literaturnaya Gazeta* last month published a round table discussion on the role, status and career prospects of Soviet engineers.

One complaint was that since 1948, engineers' salaries had risen by an average of 20 per cent in real terms, while those of "ordinary" workers had doubled. The participants also noted that young people seem reluctant to apply to engineering colleges. Many of the most prestigious higher technical schools, such as the Leningrad Mining Institute, no longer set competitive entrance examinations, while



The bicentenary of the birth of the Victorian scientist David Brewster was celebrated last week in a one-day symposium held at the Royal Scottish Museum in Edinburgh. Brewster's main claim to fame was his invention of the kaleidoscope, but he also made important contributions to work on the polarization of light. He later became Principal of the Universities of both St Andrews and Edinburgh, and was a major force in the establishment of the British Association for the Advancement of Science.

in a certain "fashionable" mathematics and mechanics faculty, the ratio of applicants to places was only 1.2 compared with 19.0 in a similar trade institute.

The impression that applied projects now take precedence was reinforced by last month's award of state prizes for science and technology. The projects honoured included work on urban water supplies from underground sources, the enrichment of desert pastures, bone transplants for diseases of the jaw and the future development of the fuel and mineral deposits of the Kansk-Achinsk basin. Even an apparently theoretical study of high temperature plasma was, according to Professor Nikolai Arzhanikov, the secretary of the awarding committee, "a major contribution" to the development of thermonuclear power.

The yearly stocktaking of Soviet life and progress which follows the Revolution Day parade included extensive press discussions of how to accelerate the implementation of research results in Soviet industry. Dr Boris Paton, president of the Ukrainian Academy of Sciences, suggested that the concept of "implementation" might profitably be deemphasized and the stress placed on "cooperation" between science and industry. The distinction is not trivial; enterprises which have an active role in developing new technologies, rather than simply "implementing" the results produced by some research institute, will be eligible to share in major Soviet prizes.

Whatever the form of the link between science and industry, however, it ultimately depends on an appropriate supply of scientists and technologists. During the past decade, science planners have concentrated on linking universities

and technical institutes into regional "science centres" whose research, it has been urged, should serve the needs of local industry. Obraztsov's complaints of the plight of universities suggests that too little attention has been paid to their major function as teaching and training establishments.

Vera Rich

#### Clean air legislation

#### Forces massing

Washington

Indecision within the Reagan Administration about how to reform the Clean Air Act is playing directly into the hands of environmentalist groups and their congressional supporters who feel that the act should be strengthened rather than weakened. It is also upsetting industry groups who had looked to the new Administration to reduce the burden of environmental and health regulations.

The current law was passed in 1970, and must be renewed by the Senate before the end of the year. The debate on revising the law is regarded as the first major test of President Reagan's determination to pursue his election promises of regulatory reform.

Even some members of the Carter Administration have argued the need for significant changes in the act. Last week the Brookings Institution in Washington published a paper by economist Lester B. Lave and Dr Gilbert Omenn, previously associate director for health sciences in the Office of Science and Technology Policy, which is sharply critical of current air quality legislation.

Claiming that the main reason the

nation's air has become cleaner in recent years has been the switch to cleaner energy-producing fuels, Dr Lave and Dr Omenn propose that the act should consider individual differences in susceptibility, as well as the availability of personal preventative health measures, when determining the acceptable "remaining risk" resulting from the various levels of proposed controls.

Industry itself is pushing for even greater changes. Public utilities, for example, are strongly resisting efforts to write into the act significant extra controls on emissions from power stations aimed at reducing the problems of so-called "acid rain" formed when sulphur dioxide reacts with atmospheric moisture.

How much is eventually changed, however, depends largely on the results of debates within the Senate Environment and Public Works Committee. And so far the lack of a clear battle plan from the Administration has left the initiative with those fighting to maintain the status quo.

The level of permissible emissions from motor vehicles has been one of the most contentious parts of the 1970 legislation. At the time, Congress set tight numerical limits on automobile and other vehicle emissions, with a strict timetable for their implementation, although under pressure from the automobile industry the deadlines have been gradually put back.

In their report for the Brookings Institution, Dr Lave and Dr Omenn argue that Congress should not be involved in setting detailed regulations for motor vehicle emissions because of the unnecessary rigidity that results. Instead, they say, Congress should delegate more, although still retaining the oversight role.

The Senate committee does not agree. For example, Environmental Protection Agency (EPA) assistant administrator Kathleen Bennett told the committee that, because of the expense involved, the Administration proposed to lower from 90 to 60 per cent the reduction in truck hydrocarbon and carbon monoxide emission required by 1984. But Senator Gary Hart replied that the figure of 60 per cent had been "plucked from the air" and argued that EPA should not be making economic decisions on public health issues.

Within the Administration, much of the blame for the current disarray in EPA's strategy is being placed on Administrator Mrs Ann Gorsuch, who announced in August that she would not be seeking major changes in the act, but was rather pushing for the inclusion of several basic principles on which future regulation should be based (Nature 13 August, p. 574). The result has been serious concern in several industries that the Administration is failing to fulfil its election promises. At the same time there is relief among Republican congressmen who already feel the worsening economic situation is jeopardizing their chances of re-election next David Dickson

#### Commercial rocketry

#### One for Ariane

Ariane, the European satellite launcher, has at last won a commercial place in the United States market. Last week, Arianespace, the French-based company which manages commercial exploitation of the European rocket, signed a firm \$50 million contract with the US General Telephone & Electronics (GTE) Satellite Corporation to launch two telecommunications satellites in 1984. The order, the first that Arianespace has won from an American customer, seems to confirm at least some of the fears of US space officials that Ariane could take business from the National Aeronautics and Space Administration, which until now has enjoyed a virtual monopoly outside the Eastern bloc for launching heavy satellites.

Ariane's chief attraction for US customers, who enjoy a 25 per cent discount, is its relatively low cost. At about \$25-\$30 million per satellite, an Ariane launch is 15-20 per cent less than a launch on a Thor-Delta rocket. Delays and uncertainties over the space shuttle have also made Ariane more competitive. Arianespace is optimistic that last week's deal will be followed by others.

Arianespace officials seem pleased with their own marketing efforts and those of the Grumman Corporation, their US agents. Twenty-one satellites from twelve customers, mainly European, have so far been committed for launch on sixteen Ariane flights. Three firm contracts, including that with the GTE Satellite Corporation, have been signed and sealed

since the third successful Ariane test flight last July. In addition, ten potential customers hold reservations for launching sixteen satellites on nine Ariane flights.

The order books are as healthy as can be expected after only three out of four test flights, according to Arianespace. Their future health, however, will depend on the fortunes of the fourth test flight scheduled for 18 December.

Judy Redfearn

#### European cooperation

#### **COST** in limbo

Brussels

The celebrations last week marking the tenth anniversary of COST, an organization pursuing "European Cooperation in the Field of Scientific and Technical Research", were appropriately low-key. Indeed, the role of COST itself has become so unobtrusive that its officials fear that its future is endangered by widespread ignorance of its purpose and even of its existence.

The chairman of the COST Senior Officials Committee, Johan Martin-Löf, speaking to the small group of senior officials and diplomats which had gathered to celebrate the anniversary, did his best to emphasize COST's solid achievements: that 35 different research projects had begun in the past decade, ten of them had been completed, and that this had involved ten EEC member states and nine other West European countries, all for a small extra outlay.

Part of COST's problem is that it is not a conventional international organization but a club which invites member countries to share the results of usually public

research. Once a project has been agreed by the intricate committee structure, individual member countries select the aspects they wish to work on and by financing only its own share of a project, each participant country has the right to the results of the entire research effort.

COST's vague status has complicated the administrative problems of coordinating the research programmes of 19 countries. A small secretariat in Brussels is its only direct physical manifestation. A major obstacle for those taking part in COST projects is the financing of travel. And Martin-Löf admits that "the time it takes to handle even relatively simple matters is often excessive". A further problem is the lack of money for the administration of projects once launched.

Suggestions for new projects have to come from the member states themselves and the most dynamic field for new projects seems to be in telecommunications, transport and materials science. Last week, a memorandum of understanding was signed for the COST project on high-temperature materials for power plants.

Jasper Becker

#### Asian ecology

#### Loss of balance

Bangalore

The increasing demand for wood for fuel in Nepal and Thailand is threatening the ecological balance of a large part of South Asia. In Nepal, deforestation is so rapid that it has been estimated that the country will be without forest by the year 2000.

The lack of tree cover is already causing two major problems. First, the once fertile valley lands are losing their ability to assimilate rainwater, causing a decline in vegetation, fast-spreading aridity and bird migration. Second, an estimated 240 million cubic metres of top soil is being carried into streams and rivers flowing into India and Bangladesh.

It was not until 1976 that the Nepalese government acknowledged the magnitude of the danger. It has now launched a 400 million rupee project, to be financed by the International Development Agency and other organizations, which aims to convert nearly 50,000 hectares of land into community forests over the next five years to meet the growing need for firewood.

In Thailand the government is also undertaking a replanting scheme concentrating on high-yielding rubber trees. The old unproductive rubber trees are to be used as construction materials in a plan which should, says the Thai Agriculture Ministry, bring in around \$60 million. Trials have shown that trees can be sprayed with chemicals and conserved at plantation sites for up to two years — at present more than 80 per cent of old rubber trees are felled and burned in a haphazard manner. Agricultural scientists have also examined the possibility of using the old trees for B. Radhakrishna Rao pulp.

#### Harsh sentence for Paritskii

A Khar'kov court has sentenced Aleksandr Paritskii, a 43-year-old specialist in electronics, to three years in a labour camp. The sentence was the maximum possible for his formal offence — disseminating anti-Soviet propaganda. Its severity may be due to one of Paritskii's activities which was not mentioned in the formal charge — the organization of a weekend "university" for young refusniks and the children of Jewish activists.

Exclusion from higher education is one of the regular sanctions imposed on those Jews caught in the limbo between applying for an emigration visa and actually being allowed to leave the country, and a fortiori on those formally refused permission to leave. Although since 1973 an increasing number of unofficial seminars have been organized for refusniks excluded from employment in the academic professions, until recently nothing formal had been done at undergraduate level.

In autumn 1980, however, Paritskii and a handful of fellow activists in

Khar'kov founded their "refusniks' university" with 25 students. Structured courses were held every weekend, throughout the academic year. Mounting harassment of Paritskii, followed by his arrest, has made it impossible for the university to reconvene this year.

The absence of the university from Paritskii's charge-sheet is interesting. Paritskii's colleagues from the "university" were not even called in for interrogation in connection with his trial. This may well mean that the authorities fear that an open attack on the "university" may simply result in a similar initiative springing up elsewhere and hope that with Paritskii gone, the "university" will simply disappear. Certainly the authorities seem to be interested in undermining his academic reputation. Earlier this year, he was one of three refusnik scientists who, in a move unprecedented in the Soviet Union, were deprived of their "Candidate" (PhD) degrees because of their political views. Vera Rich

#### Theory of evolution

#### Huxley speaks up

Sir Andrew Huxley, his grandfather's grandson, startled the Anniversary Meeting of the Royal Society this week with a stout defence of the theory of evolution. Traditionally, presidents have used these occasions for making gentle complaints about the British government's handling of science and education. But Huxley, starting with Sir Edmund Leach's statement at the British Association meeting this year (see *Nature 3* September, p.19) that many of Wilberforce's nineteenth century criticisms of Darwin were now accepted, said "here is a Huxley answering the challenge".

The correspondence in the past twelve months about cladistics and the British Museum (Natural History) still seems to rankle. In defence of cladistics, Huxley said that even the insistence of "transformed cladists" that taxonomic classifications should be made without reference to "necessarily conjectural" ideas about evolutionary processes and

#### University problems

Among the points in the secular part of Sir Andrew Huxley's anniversary address on Monday were the following:

- The dual support system for academic research, "a shadow of what it used to be", is in danger of being totally obliterated.
- Although the British government had agreed in its expenditure white paper in March to protect basic research, research councils are having to spend more on making good deficiencies of university budgets and are thus less able to support new research projects.
- There is a danger that universities will respond to this summer's budget cuts by further reductions of support for their own research.
- If a university "shows that it is moving in the right direction", the British government should be prepared to reduce the speed or the severity with which cuts are required.
- On the increased fees for overseas students, "I wish to add my voice to the many who have expressed dismay at this drop in our contribution to development, and at the loss of goodwill towards Britain that this policy will entail in the long run".

The society's annual report, also published this week, records that its own subvention from the British government increased to £4.2 million in the year just ended, from £3.7 million the previous year. General administration cost £564,000 — the rest was spent on support for people, international exchanges, grants for research and on international subscriptions.

selective pressures "is in no sense a denial of evolution".

Huxley said that his reaction to the correspondence in *Nature* had been to wonder whether he should "take up the cudgels on Darwin's behalf", but that he had been "mollified" by a "very sensible editorial article" in *Nature* (30 July, p.395). His concern now was to counter the "ripples [which] continue to spread".

In passing, Huxley said that the calculations of Hoyle and Wickramasinghe (see Nature 12 November, p.105) suggesting that there could not have been enough time since the origin of the Universe for life to have evolved could be "dismissed quickly". He said that the characteristic large number used by the authors (1040,000) represents the chance that 2.000 enzyme molecules would be formed simultaneously "on a single specified occasion". "Since we know neither the nature of the hypothetical self-replicating system nor the composition of the primaeval soup", the conclusion is, however, "wildly uncertain".

It is unlikely that Hoyle and Wickramasinghe will accept their dismissal on these grounds. Their arguments are based on the "information content" of the evolved genome, and on estimates of the rate at which information content might plausibly accumulate.



Huxley in defence of evolution

On the doctrine of Gould and Eldridge of punctuated equilibria, Huxley said that the debate lies within the Darwinian framework but that its authors have exaggerated the suddenness with which new stable species emerge. In any case, he said, Darwin's own guess that it would take ten or a hundred thousand generations for two "well-formed" species to emerge from a single species is not inconsistent with the doctrine of punctuated equilibria, while later editions of The Origin of Species, like the writings of neo-Darwinians such as G.G. Simpson, contain statements which are "exactly equivalent" to punctuated equilibria. In the circumstances, Huxley said, he could not understand how

#### Rhesus monkeys die

The University of Birmingham is being forced by financial and legal circumstances to kill most of its once thriving monkey colony.

Unable to raise the £250,000 needed to bring its monkey housing up to present safety standards, the university has for the past two years been exploring the possibility of dispersing all the animals elsewhere. Although there is no shortage of willing recipients, the university has finally decided that the probable legal risks in dispersing its adult monkeys are too great to delay any longer what they see as the almost inevitable slaughter of the animals.

The legal difficulties arise because almost all the adult rhesus monkeys have been used for experimental purposes under Certificate B of the Prevention of Cruelty to Animals Act which demands that animals be killed at the end of an experiment. Many experiments on the Birmingham monkeys were in midstream when they were stopped by the university pending modernization of the housing.

Since the experiments were interrupted rather than finished, it has been argued that the monkeys could be transferred elsewhere and experimentation continued. But the university, in consultation with the Home Office, has decided that it would be too risky to test that interpretation of the law.

Peter Newmark

punctuated equilibria could be regarded as contrary either to Darwin's own ideas or to those of neo-Darwinism.

On Sir Edmund Leach's echo of Wilberforce's complaint that the fossil record lacks evidence of the "missing links" between major stable groups of species, Huxley replied with a list of "missing links" whose discovery "has gone on at an increasing rate since before the publication of *The Origin of Species* to the present day", such as *Archaeopteryx* (1861), the fossil horses and hominids more ancient than the Neanderthals.

Huxley went on to dismiss other complaints made by Wilberforce and Owen a century ago, chiding anti-evolutionists for overlooking the "positive indications" from biochemistry, which have demonstrated the "ubiquity of the main metabolic pathways" and of Mendelian inheritance — "precisely what Darwin needed".

Likening the present status of evolution to that of biochemistry fifty years ago, Huxley listed the remaining "gaps" that seem to him important — the absence of evidence for the origin of the major groups of animals in the Palaeozoic, the inheritance of interspecific sterility, the role of genetic drift, the origin of life and the existence of consciousness — "too often swept under the carpet".

## RRESPINDENCE

#### Hoyle on life

SIR — Surely there is some mistake in your summary of Hoyle's contention that the Universe has not been here long enough to permit the evolution of life forms of the complexity found on this planet (*Nature* 12 November, p.105).

It is true that the probability of chance assembly of the system of genes upon which our life system depends is very low. However, it is not this particular probability which is relevant, but rather the result of multiplying this admittedly small number with the very large number representing the total number of gene systems which could also be the basis for complex life forms.

Any particular gene possesses its enzymatic properties as the result of its configuration in some quite small part of its total chain length. Although the chance of random assembly of the total chain, specified at every point, is small, the chance of assembly of a gene specified over a small fraction of its length is obviously much larger.

If you modify your account of Hoyle's arguments along these lines, I suggest you will conclude that we are all here after all.

A.E. ROUT

British National Oil Corporation (Development) Limited, Glasgow, UK

#### **Postdocs in Canada**

SIR - I would like to pass on some news that I have just obtained with regard to the status of foreign postdoctoral fellows working in Canada. The Canadian Immigration Regulations require that before a foreigner can be offered employment, the position must be advertised through Canada Employment Centres, and suitably qualified nationals must be given preference. This regulation does not apply to postdoctoral fellows, but Canadian officials both in London, England and in Toronto assured my wife and myself that it did apply to their spouses. When we came to Canada last year my wife was unable to find a job, and since postdocs in biological sciences are not well paid (see Nature 11 June and 8 October), it became necessary for my wife to return to England to work.

This seemed an unfair system, so I brought it to the attention of Lloyd Axworthy, the Canadian Minister of Employment and Immigration. I have received a reply from him to the effect that since such restrictions do not apply to Canadian postdocs going to work in Britain, Canada is prepared, in this case, to reciprocate. He is instructing all Canadian immigration officials that this is now the case. So in the future, married postdocs will be able to move between both countries without facing financial hardship. However, it is implied that this relaxation does not apply to individuals from countries which do not already show hospitality to Canadians. I am impressed by the fact that the experience of an individual can successfully modify governmental regulations, but surely it is opportune, and for the mutual benefit of all concerned, for all countries which wish to foster academic exchange to take steps to relax immigration rules for the families of postdoctoral fellows. DYLAN EDWARDS

The University of Western Ontario, London, Canada

#### **Open relations**

SIR — Your article about the agreement between this hospital and the international chemical company Hoechst AG (Nature 5 November, p.5) states that the Government Accounting Office forced open the contract heretofore kept from Congressman Albert Gore. In fact, Massachusetts General Hospital gave the document to Congressman Gore on 21 July 1981.

What we did in October was to make the contract available to the general public from whom we have had a number of requests. In fact, we know of no other instance in which such a contract has been made available to the public. We have taken this course because we are presenting the agreement to our external Scientific Advisory Committee this month. The committee's topic, scheduled many months ago, is scientific relations with industry.

JOSEPH B. MARTIN Massachusetts General Hospital, Boston, Massachusetts, USA.

#### Life out there

SIR - It was interesting to read in iuxtaposition Dr Zuckerman's review of the extraterrestrial issue and Professor O'Neill's review of Rood and Trefill's new book on the same subject (Nature 5 November, pp.10, 25). One is struck again by the fascination - not to say fixation - that earlier and "young revolutionary" students of this issue have had with technology as an index of "advanced" civilization. The idea that other planets may have chosen to focus on spiritual rather than technical development dates back to at least the eighteenth century polymath Swedenborg. As is often noted nowadays, the validity of technology - rather than, say, spirituality as an index of cultural achievement is less than clear even for our own globe. It seems premature to disregard the Drake (or Green Bank) equation simply due to lack of verification of its technology/colonization components. What is called for instead, perhaps, is greater conceptual bandwidth in its KURT SIMONS formulation. Wills Eye Hospital, Philadelphia, Pennsylvania, USA

#### Leave links open

Sir — Dorothy Hirsch, Executive Director of the Committee for Concerned Scientists, ended her eloquent letter (Nature 13 August. p.578) about the plight of Soviet scientists with the charge to us that "We cannot afford to become hardened to the plight of oppressed colleagues who have much to contribute to a vital international science". Time (28 September) reports that exiled Soviet writers, despite enormous grievances against the Soviet government, have pleaded that free world publishers should not boycott the Moscow International Book Fair which means so much to the ordinary Russian citizen as well as the Russian literati. I have just returned from an international medical/scientific congress in Moscow, and wish to make a similar appeal to Western scientific groups resolved to boycott all scientific interchanges hosted by the Soviets.

At that congress I concluded my scientific presentation with a slide listing a dozen of the Soviet scientists sentenced to "internal exile"

(imprisonment) for their dissident views, and stated simply that my presentation was dedicated to "these fellow scientists in the sincere hope thay they may be free again some day soon to join with their colleagues from other nations in open scientific interchanges such as this one". It is still tearful for me to recall the response of the stony-faced Russian scientists in the audience to that statement. Almost in unison, they lowered their eyes, nodded very slightly, and a whispered chorus of "Da! Da!" ("Yes! Yes! Yes! Yes!") filled that Moscow meeting room.

I appeal to individuals and scientific societies that have decided to boycott Soviethosted scientific interchanges as a protest that they reconsider their decision. Boycotts do little to embarrass or punish the Soviet government for its repressive and reprehensible policies towards dissident scientists (writers, intellectuals, etc.); attendance at these scientific interchanges as representatives of free world countries may do much to assure the Russian scientific/ academic community of our awareness of their plight, and of our deep concern and sympathy for them. It lets them know of our resolve not to cut them off from new information from the outside world, but to share with them our scientific findings, our ideas, our literature, which they, like us, should not be denied. They are obviously not optimistic that things will change in the near future. Let us at least continue to help them endure what they must so that they can continue with their careers, with their scientific research which they love as much as we do. LEE FRANK

Department of Medicine, University of Miami, School of Medicine, Miami, Florida, USA

#### Not cricket either

SIR — Certainly the City of New York's Yankees deserve numerous boos and catcalls for their performance in the World Series. Just as certainly, your publication deserves similar treatment for printing a column ("Shame on New York", Nature 5 November, p.2) demonstrating both little understanding of the subject and a shameful neglect of facts evident to all but the most illiterate American youth.

You state "Part of the reason why the Yankees lost is that they were not as skilled at catching and throwing the ball as were the Dodgers", a glaring misstatement in light of the Dodgers' second baseman's infamous accumulation of six World Series records for "errors committed" (literally: failure to catch and throw the ball). In addition, claiming that there was "little to choose between the batting performance of the two teams" ignores the dismal failure of one of the Yankees' most depended-upon batters (Dave Winfield, playing under a multi-million dollar contract) to connect for more than one hit in twenty-two at bats during the series.

Concerns for propriety and my own temperament forbid me to further chronicle and refute the errors contained within the column. Allow me to end my tirade by pointing out your insensitivity in measuring the speed of a baseball in units of ''metres per second'' thereby violating the worshipful reputation of baseball as ''a game of inches''.

The Salk Institute,

Peter Syka

San Diego, California, USA

## NEWS AND VIEWS

# Consider the incompleteness of the geological record

from Tjeerd H. van Andel

For more than a century geologists have felt comfortable with the notion that geological processes, although very slow, are also steady and so capable of moulding the Earth given enough time. The steady drip hollows the stone. The idea of steadiness came, at least in part, in reaction to the catastrophist view of the early 19th century which saw the history of the Earth as punctuated by brief but violent upheavals separated by long times of unchanging tranquillity. The slowness was suggested by the difficulty of observing many geological processes at work, and was enhanced by the growing awareness of the great length of time that was available. The rise of a continent may not be visible in a lifetime, nor the removal of a mountain by erosion, but the 4.5 billion years of Earth history provide enough time for all recorded events.

In recent years we have learned that many, perhaps most, geological processes are more rapid than once believed. The Atlantic Ocean widens at a rate of 20 km per million years, the rise of sea level at the end of the last Glacial locally pushed the coast inwards at a rate of 3 km per century. Every year the Mississippi delta adds 50m of land to the state of Louisiana and, if left undisturbed, the equatorial ocean would fill with sediment in a few hundred million years. If these rates are typical, do we need all of the available time to account for the events recorded by the rocks?

The answer is clearly negative. Studies of modern depositional environments taught us the rates of deposition typical for each. We may apply these rates to the deposits left behind by their equivalents of the past if we make allowance for such matters as compaction due to overburden and time. For years I have been intrigued by the result of such estimates. In Wyoming, a sequence of early Cretaceous sandstones and shales closely resembles the coastal sediments of the present Gulf of Mexico. Applying the appropriate rates of deposition we find that a mere 100,000 years would suffice to produce the entire sequence. Yet the stratigraphical interval occupied by the deposits is quite firmly known to encompass about 6 million years. We may repeat the experiment elsewhere; invariably we find that the rock record

requires only a small fraction, usually 1 to 10 per cent, of the available time, even if we take account of all possible breaks in the sequence. Evidently deposition, unlike work in Murphy's law, does not expand to fill the time available. This might in principle be expected but the universality and especially the magnitude of the shortfall are startling.

This fascinating observation has attracted the interest of a few geologists but only in passing. Recently, however, it has inspired Peter M. Saddler to examine the issue in depth (Journal of Geology 89; 569, 1981). If it is indeed true that ancient sediments represent less time than available, the accumulation rate obtained by dividing the thickness of a deposit by the length of the interval it occupies ought to be smaller than the deposition rate measured directly in the equivalent modern environment. This, indeed, turns out to be the case and the fractions of time not represented by deposits are very large. Even more interesting, the massive amount of data put together by Saddler shows that accumulation rates (thickness/available time) are inversely proportional to the length of the interval over which they are estimated. This inverse relationship holds true for all kinds of environments, even though the precise form varies.

Because one can resolve very short intervals only in young deposits while very long ones carry us far into the past, one might surmise that what we are observing is a progressive increase in the rate of deposition towards the present. This assumption, unattractive for several good geological reasons as well, is incorrect because the inverse correlation exists between the accumulation rate (or the thickness of the preserved sediment) and the duration of the observed interval, but not its age. Thus it appears that indeed the geological record is exceedingly incomplete, and that the incompleteness is greater the shorter the time span at which we look.

Tjeerd H. van Andel is professor of oceanography in the Geology and Geophysics Departments at Stanford University, California.

It does not startle us that the geological record is damaged by gaps; we have known that for a long time. Nor is it surprising that the amount of time represented by breaks is significant. Some time ago, before the deep-sea drilling programme of the Glomar Challenger, it was widely believed that the sediment record in the deep ocean should be very complete because the environment is quiet and is the ultimate resting place of all debris. This turned out to be far from true. In the South Atlantic. for example, barely half of the history of the last 125 Myr is recorded in sediment. It is no better in other oceans and surely worse for shallow marine and continental environments. Even so, the level of incompleteness with which we are concerned in this paper is far in excess of what has been observed or might reasonably be anticipated.

If erosion and other ravages of time are the cause of the missing record, one should expect the incompleteness to increase with age. This, however, is not the case and the explanation cannot be so simple. Saddler's voluminous data show that shortfalls do not only occur on all time scales but that they are a function of the time scale. Taking a hypothetical fluviatile sequence 500 m thick and spanning 7.5 Myr, he shows that a reasonable annual deposition rate, in the flood plain of a permanent river for example, will produce this thickness in 5,000 years. The observed sequence would thus be 0.075 per cent complete. If, on the other hand, we use a deposition rate on an hourly scale, a flash flood perhaps, the section's completeness would be 1 in 10 million.

The consequences of the inferred severe incompleteness of the geological record and its relation to time scale deserve our most serious attention because they affect the history of the Earth in many ways. Consider, for instance, the desire to locate the real boundary between two stratigraphical rock units. The likelihood that such a boundary will have been preserved is much greater for units of long duration than for brief ones: our chance of finding the boundary between two strata of 5 Myr duration in an interval of 25 Myr is much greater than that of locating the contact between two beds each spanning 1

Myr. Conversely, if we believe that we have located the deposits associated with a brief event, an asteroid impact perhaps, in several stratigraphical sections, we ought to consider the extremely low probability that any event on a decadal time scale would be preserved at all, even once.

Many of the gaps in the record can be clearly seen, especially those on longer time scales, but the conclusion is inescapable that most breaks must go unnoticed. The geological record appears to consist of brief periods of activity separated by long times during which nothing happens or, alternatively, long times during which brief events leave minimal imprints which are removed by the next ephemeral event. However, even if only the occasional major event leaves a record substantial enough to be preserved, one would still like to think that the intervening times of erosion and non-deposition might leave some trace, a subtle alteration of the surface, a bit of soil formation, some burrowing. There is plenty of evidence that such effects exist and can be observed, yet most gaps must remain unmarked. I was much influenced early in my career by the recognition that two thin coal seams in Venezuela, separated by a foot of grey clay and deposited in a coastal swamp, were respectively of Lower Palaeocene and Upper Eocene age. The outcrops were excellent but even the closest inspection failed to turn up the precise position of that 15 Myr gap.

The geological record may thus be a record of rare events separated on any time scale by numerous and long gaps. Apart from the problems this presents in stratigraphy, there is no more cause for disquiet in this concept than there is in the atom model, also an object of little substance and much space. If the history of the Earth is mainly shaped by rare but major events, we need not greatly regret our inability to track the trivial day to day happenings. Intuitively, the notion seems plausible: nature all around us seems to change mostly by leaps. If at first sight such a record of history appears uncomfortably limited, like one of only battles and kings, two centuries of study have shown that it makes sense even in this abbreviated form.

There are, however, also disturbing aspects beyond the need of a vastly increased care in stratigraphy and chronology. Among the many ideas fermenting today in the study of evolution there is one, frequently heard, that ascribes the major evolutionary steps to a jump advance, a concentration of major change in a very brief interval of time. There seems to be no good reason why such pulses of evolutionary change should coincide with the major rare events that built the sedimentary sequence in which the record of evolution is contained. Thus, the new 'catastrophist' view of the sedimentary record implies that key elements of the evolutionary record may be forever out of

# A potpourri of plucked patches

by Henry A. Lester

HAMILL, Marty, Neher, Sakmann and Sigworth<sup>1</sup> and Horn and Patlak<sup>2</sup> recently described new techniques for improved recording from single ion channels in biological membranes. They also showed how to excise the membrane patch under study, so that the experimenter can manipulate the solution bathing either the cytoplasmic or the external face. These methods, described in a *News and Views* article by McBurney<sup>3</sup>, are leading to an explosion of new knowledge. The pace can be gauged by this review of six months' progress.

Studies on acetylcholine receptor channels have led the way. Two papers in this issue of Nature reveal that channel behaviour is more complicated than the former view of a simple transition to a unique open state with a smooth, featureless ion flux. First, Hamill and Sakmann (see p.462) shows that rat myotube channels have two open states that differ by several fold in their conductance. Channels open to the 'main' state, then hop (with a low probability) to the less conductive 'substate'. From the substate they can either close or return to the main level, with roughly equal probabilities. In addition to this wrinkle in the description of the open channels' conductance, the improved temporal resolution of the gigaseal technique has enabled studies on the fine structure of the gating events. The opening and closing transitions themselves require no more than 10µs so that complete events lasting just  $50-70 \mu s$  can be resolved. Colquhoun and Sakmann (see p.464) now report that the open state is indeed interrupted by events of this brevity when the agonist is suberyldicholine in frog muscle. On the average, a single open 'burst' is interrupted 3.1 times by gaps whose lifetimes are exponentially distributed with a mean of 150  $\mu$ s. The investigators consider the possibility that these Nachschläge represent the longsought transitions between the bi-liganded open and bi-liganded shut states. An additional, more subtle form of 'openchannel noise' can be observed in the patch-clamp records of other investigators. Such fluctuations probably do not arise at the single-ion level, but may be explained by motions of the amino acid side chains that presumably line the channel. If so, patch-clamp recordings provide an elegantly simple way to obtain information on the molecular dynamics of proteins.

The glutamate receptor channel of locust muscle weighs in with a hefty conductance of 125-150 pS. Recent patch-clamp investigations have sought to understand the basis for the sigmoid dose-response curve. With an elegant multibarrel internal

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perfusion system for the patch electrode, Cull-Candy et al.<sup>4</sup> found that the channel opening rate increases with the square of the glutamate concentration, suggesting that two glutamate molecules are required to open a single channel. Gration et al.<sup>5</sup> found that the mean channel duration also increases with [glutamate] as though all sites must be vacated to allow channel closing. It is not clear whether these two factors are compatible with each other; it is perhaps relevant that Gration et al. observed non-random features in the gating kinetics, suggesting as yet unexplained transitions in agonist affinity.

Electrically excitable Na+ channels are rather more difficult to study carefully because the open conductance is only 6-18 pS; depending on the conditions. Nonetheless it seems clear from the available recordings that the open Na+ channel does not flicker<sup>6,7</sup>. Horne et al. 7 compared the closing of channels which had opened either early or late during a depolarizing clamp pulse and obtained evidence for the view that activation and inactivation are independent processes, as in the original Hodgkin-Huxley formulation. However, macroscopic voltage-clamp experiments have for some time pointed to discrepancies between observed kinetic behaviour and that predicted by the Hodgkin-Huxley equations; and patch-clamp investigations now confirm that the open state lasts longer than would be predicted if the channel could be closed with equal probability by transitions in any of three putative subunits. Apparently the closing rate is limited by the last transition preceding the open state in the activation pathway, a conclusion that is particularly clear when the inactivation pathway has been removed by exposure to N-bromoacetamide8.

In almost every type of cell that has been patch-clamped, the investigators were startled by enormous signals that took the oscilloscope trace off screen. Upon further investigation these signals were recognized as Ca2+-activated K+ channels (chromaffin cells9, myotubes10, autonomic ganglia11, Helix neurones19 human erythrocytes<sup>12</sup>). These channels have conductances of 100-200 pS and show very interesting flicker and open channel noise. The opening rate increases with both [Ca2+] and depolarization, with an absolute requirement for Ca2+, as though the membrane potential is influencing the effective affinity constant for Ca<sup>2+</sup>. It would be most interesting to know (a) the Hill coefficient for the doseresponse curve and (b) whether the channel still functions after procedures that remove calmodulin from the membrane.

Other potassium channels have been observed with the patch clamp. Ohmori et

al. 13 recorded inward ('anomalous') rectifier channels on myotubes as they flickered due to the binding and dissociation of Ba2+ ions. conductance (10pS), kinetics and voltage sensitivities agree well with results from noise and voltage-jump relaxations. However, at the recent Neuroscience meetings H.C. Hartell reported that muscarinic agonists induce surprisingly brief K + channels in cultured heart muscle. The average duration is 37ms or about a tenth as long as the time constant derived from fluctuations14, voltage-jump relaxations<sup>15</sup>, or concentration-jump relaxations<sup>16</sup>. The channel conductance is 8pS. Hartzell speculates that the slow macroscopic relaxations derive from repeated binding of agonist to receptors, leading to multiple channel activations.

There's more. Bormann et al. 17 recorded

single GABA-sensitive channels in excised membrane patches from cultured spinal cord neurones. The channels are selective for chloride and have a conductance of 23pS. And Lux and Nagy<sup>18</sup> observed single Ca2+ channels in Helix neurones. The opening rate increases with depolarization; the channel duration (about 25ms), on the other hand, shows no voltage dependence. The conductance of 5-15 pS is much larger than expected from noise measurements but exact comparisons could be misleading because the Ca2+ channel is not ohmic.

Patch-clamp recording are so easy (compared with conventional voltage clamp experiments), and so immediately gratifying, that we are incorporating them into an undergraduate laboratory course at Caltech. The only specialized instruments required are a high-quality microforge and a carefully engineered headstage (now

available from several manufactures). Data analysis is tedious and time-consuming, but this will undoubtedly not prevent a host of interesting and important investigations with the new techniques.

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# T lymphocytes: repertoire and recognition

Any discussion of T cell immunology sooner or later leads to controversy. Although T cells, like B cells, are capable of recognizing foreign antigens with exquisite specificity, the nature of their activation is far less well understood. First, they are a heterogeneous population pursuing both effector and regulatory functions, recognizing mainly membrane-bound antigens, and second, they are restricted to seeing antigens in the context of molecules of the major histocompatability complex (MHC). This has been one of the most exciting findings in immunology in recent years and raises questions of how both a foreign antigen and a MHC molecule in the same membrane can be recognized by a single T cell and why activation results only when both are present. Whether one or two receptors are required for the recognition step is hotly debated and the mechanism by which such a sophisticated repertoire develops within each individual is also in dispute. It was with these two major questions in mind that many workers in the field were recently invited to a workshop in Rüdesheim in Germany\*.

#### Ontogeny of the T cell repertoire

The T cell repertoire is constrained both by the requirement of MHC restriction and by the need to remove or inactivate, early in development, T cells capable of responding to normal antigens found on the cells of the individual. This 'learning' process has been believed to occur in the thymus but recent studies of the congenitally athymic nude mouse are raising some questions. Nude mice have T precursor cells but the development of the repertoire is deficient. H. Wagner (Johannes-Gütenberg University, Mainz) and T. Hünig

(University Würtzburg) reported the generation in vitro of cytotoxic T cells from precursors found in both normal and nude mice, but the nude mouse was deficient in precursors against certain antigens. J.C. Cerottini (Luding Institute), using similar techniques, found only quantitative differences between the two animals although nobody has yet been able conclusively to demonstrate functional helper T cells in the nude mouse. The precise immunological status of the nude mouse is therefore still not clear but the existence of at least some precursor T cells which can be stimulated in vitro suggests that some pre-thymic development of the repertoire may occur. A. Singer (NIH) described experiments specifically designed to test this hypothesis. He constructed chimaeras using an  $(A \times B)$  F. host, thymectomized, lethally irradiated and grafted with an A thymus and A bone marrow, whose recovered thymocytes were shown to be tolerant to B MHC determinants. This suggested that tolerance had been induced prethymically and some pre-T cells at least must already have learned self MHC. Support for extrathymic development of some precursor T cells also came from experiments reported by R. Miller (Ontario Cancer Institute, Toronto) who described a novel culture system for generating T cell colonies from single immature precursor T cells which were MHC restricted but surprisingly developed several antigen specifities.

On the other hand, transplantation experiments have shown that the thymus does exert a significant but as yet unclear effect on the generation of the T cell repertoire. It is thought that the thymic epithelium may act by presenting antigens and self-MHC molecules to T precursor cells after which selected clones are stimulated to develop to maturity. I. Weissman

University) presented provocative data on the discrete distribution of different classes of MHC molecules in different regions of the thymus, but there is as yet no firm evidence of a common pathway by which T cells pass through the thymus and undergo step by step acquisition of the repertoire in the normal animal. Clearly new approaches are required and R. Jordan (Newcastle University) presented interesting preliminary experiments using cold treatment of thymuses in vitro to obtain pure thymic epithelium which may be useful in understanding its role in antigen presentation.

The picture emerging seems to be that the thymus acts on T cells to select precursors which are restricted to recognizing antigen in conjunction with self-MHC molecules and to expand this population, possibly by an interleukin-2 signal. Some clones, however, may develop outside the thymus without having been selected to see antigen only in conjunction with self-MHC molecules. It may be that this population does not receive an inductive signal and so remains in low numbers in the normal animal, overshadowed by the thymic-processed, self MHC-restricted population.

The experiments described by Z. Nagy (Max-Planck-Institut, Tubingen) were very interesting in this respect. He has been looking at the phenomenon of low responsiveness which is found in specific mouse strains against certain antigens. In an in vitro system he has shown that T cells from a low responder strain can respond to the antigen if it is presented by (non-self) antigen-presenting cells. This suggests that there are two compartments in the T cell repertoire, one that recognizes foreign

Nigel Williams is a member of the editorial staff of Nature.

<sup>\*</sup>A workshop on 'The role of the thymus in the T cell repertoire' was held in Ruderheim on 16-19 September 1981 organised by the Johannes-Gutenberg-Universitat, Mainz and sponsored by the Deutsche Forschungsgemeinschaft.

antigens in the context of self-MHC molecules and another that can use allogeneic MHC molecules of the species for the context of antigen recognition. However, it seems that in the mice of the low responder strain, clones capable of responding to the antigen in the context of self MHC are absent. Such experiments emphasize the importance of MHC molecules in the generation of the repertoire and raise difficult questions about the nature of antigen recognition by T cells.

#### T cell-antigen interaction

There are two opposing theories of how individual T cells recognize both MHC molecules and a foreign antigen (X) — one model requires two separate receptors and the other, the interaction antigen model, has recently been argued by Matzinger (Nature 292; 497, 1981). This model proposes that MHC molecules interact with antigen X and are presented as a single entity to a single T cell receptor. In its most provocative form it proposes that the interaction of specific self-MHC molecules with a particular antigen X will give rise to a new antigenic determinant expressed only by the interacting complex. A prediction of the interaction antigen model is that crossreactivity with allogeneic MHC + antigen Y could occur, for example if the allogeneic MHC + antigen Y interaction produced an antigenic determinant cross-reactive with those of a self MHC + antigen X interaction. Since the cross-reactive determinants are absent from the individual non-interacting components, then if the T cell recognizes both entities via separate receptors cross-reactivity would not be expected. T. Hünig (see this week's Nature, p.460) presented experiments showing that indeed a small proportion of clones of T cells specific for self MHC + X showed reactivity against allogeneic MHC + Y under some conditions. R. Schwartz (NIH) and C. Janeway (Yale University) also reported the generation of self MHCrestricted clones specific for antigen X which showed a degree of cross-reactivity with allogeneic MHC molecules.

Another prediction of the interaction antigen model is that T cells will have no particular specificity for self-MHC molecules or antigen X alone, being specific only for a combination of the two components, whereas with the two receptor model T cells should bear separate receptors for antigen X and for MHC molecules. H. Cantor (Harvard University) presented data from a T cell clone specific for self MHC + X (in this case, cow insulin) which secreted a 68K molecule into the supernatant. The molecule bound to other related insulins with a lower but particular affinity — when passed through columns each containing a different insulin, it was recovered in the same proportion as predicted by its binding affinities. A column containing the specific restricting MHC molecules failed to bind the molecule. If this molecule is part of the

T cell receptor (which is uncertain at this stage), then a degree of independence of the antigen-binding component and the component specific for the restricting MHC molecules is suggested. If the two components are indeed presented as an interaction antigen it must be of the very loosest kind. R. Schwartz interpreted these data in terms of a single receptor which has two separate binding sites with different specificities. This could possibly explain how a receptor specific for self MHC + X could recognize allogeneic MHC molecules if these were sufficiently structurally different to make contact with both binding sites sufficient to activate the cell.

Clearly we need to know a great deal more about the precise role of MHC molecules in T cell responsiveness. To this end G. Hämmerling (Cancer Research Center, Heidelberg) showed that different T killer cell clones restricted to the same MHC molecule could be blocked from responding by monoclonal antibodies to different determinants on the MHC molecule. Until we know more precisely the function of MHC molecules, the manner in which a single T cell sees both MHC molecules and a foreign antigen is likely to remain controversial and immunologists will still not know if 1 + 1 = 1 or 2.

# Receptors, channels and whole-body NMR in Mexico

from M.J. Geisow and J. Mas-Oliva

THE 7th International Biophysics Congress and 3rd Pan-American Biochemistry Congress was held in Mexico City recently. Despite the title, no dichotomy was obvious — it is now impossible to distinguish biophysicists from biochemists on the basis of project or hardware. Many topics were covered, but a dominant theme was the measurement, biochemical analysis and functional implications of ionic gradients across cell membranes.

An understanding of transduction or ion transport through biomembranes can only come through knowledge of the structure of intrinsic membrane proteins. As yet, no example exists, whether receptor, channel or carrier, where structural determination is sufficiently advanced to allow firm conclusions about mechanism. This situation was not changed in Mexico, although bacteriorhodopsin is clearly a front runner with a tentative assignment of amino acids to the 3Å analysis and extensive work on reconstituted rhodopsin systems.

Also promising is the F<sub>1</sub> structure of proton-translocating ATPase (F<sub>1</sub>.F<sub>o</sub>) reported by Amzel et al. (Johns Hopkins University) at 9Å resolution. Three large domains, related to a similar triplet by local symmetry, seem likely to reveal the location of the five types of polypeptide as well as the ligand and effector sites. The protonophoric (F<sub>0</sub>) membrane-embedded chains may consist of a hairpin arrangement of twin helices (Sebald and Hoppe, Technical University of Braunschweig). They bear a buried, invariant acidic amino acid the loss of which, by modification or mutation, halts proton translocation.

M.J. Geisow is in the Biophysics Department, National Institute for Medical Research. J. Mas-Oliva is in the Biochemistry Department, National Institute of Cardiology, Mexico City. Another close contender is the acetylcholine (ACh) receptor, reviewed by J.P. Changeux (Pasteur Institute, Paris). The minimum functional aggregate (in reconstitution experiments) consists of four glycosylated peptides:  $\alpha(40K)$ ,  $\beta(50K)$ ,  $\gamma(60K)$  and  $\delta$  (65K) in 2:1:1:1 stoichiometry and having NH<sub>2</sub>-terminal sequence homology.  $\alpha$  is labelled by AChreactive analogues,  $\beta$  by channel blockers (such as histrionicotoxin). Three molecular transitions of the complex, revealed by bound fluorescent agonists, may be associated with a minimum of three pharmacological 'states' of the receptor.

The sodium channel of neuronal plasma membranes is more elusive. Miller and Agnew (University of Colorado) have studied a  $260,000-M_{\tau}$  tetrodotoxin-binding component of the channel complex. The binding properties of this highly charged glycoprotein are particularly sensitive to the lipid microenvironment.

Working with sarcolemma, Almers et al. (University of Washington) established that sodium channels are relatively immobile in the bilayer. Their local destruction in a clamped patch of membrane by UV photobleaching leads to loss of sodium conductance for one hour. Channels are refractory to laterally applied electric fields and are considered to be 3 orders of magnitude less mobile than rhodopsin.

The strong probability that other channels and receptors are anchored to the cytoskeleton also emerged. The ACh receptor seems to be fixed so firmly in the membrane by an alkali-extractable 43,000-M<sub>r</sub> component (not actin) that the addition of exogenous lipid leads to rafts of receptor aggregates, rather than randomly dispersed molecules. Cherksey and Zadunaisky (State University of New York) used the fluorescence of propanalol

as a probe for the mobility of the  $\beta$ -adrenergic receptor in the presence and absence of microfilament-disrupting drugs and conclude that the receptor is anchored to the cytoskeleton.

A steady improvement in the quality of 'model' membranes is taking place at the same time as the successful extraction and characterization of intrinsic membrane proteins. M. Montal (University of California) has developed a technique of spreading two monolayers of protein and lipid and apposing them across an aperture to form a planar bilayer. H. Schindler (University of Basel) employs the same technology, but has discovered that natural membrane or lipid vesicles, added to the aqueous compartments, are in equilibrium with and contribute representative lipids and proteins to the surface monolayers.

The functional reconstitution of membrane proteins will allow two persistant problems to be addressed. First, what, if any, is the influence of particular lipids on protein function? and second, what role is played by protein-protein interaction in the bilayer? P.F. Devaux (Pasteur Institute, Paris) has devised spinlabelled lipid probes which covalently attach via their head groups to reconstituted membrane proteins. This achieves a significant increase in occupancy by probe of the protein-lipid boundary. Rotational correlation times of the proteins obtained in this way indicate strong lateral interactions (proteinprotein) for ACh receptor and Ca2+-ATPase and very weak ones for rhodopsin. Spin exchange between boundary probe and lipids spin-labelled with a different nitrogen isotope show that migration of lipids into the lipid boundary of rhodopsin is independent of the head group.

Lateral interactions between the intrinsic membrane proteins of the inner mitochondrial membrane were explored by C.R. Hackenbrock (University of North Carolina). Electron transfer between the various oxidation-reduction complexes and carriers appears to be facilitated by the gregarious nature of the proteins. However, although electron transfer decreases monotonically with the addition, by fusion, of exogenous lipid, it does not cease. Freeze-fracture of vesicles reveals uniform dispersion of all particles (proteins) in lipid-enriched membrane vesicles. Antibody marker techniques demonstrate that cytochromes oxidase, b and c do not migrate as complexes. In fact, sufficient diffusional motion (measured by freeze-fracture of vesicles after relaxation of an electric field) of the intrinsic proteins can occur within one turnover of reducing equivalents to render stable proteinprotein interactions unnecessary.

Another role hypothetically associated with lateral interactions of intrinsic membrane proteins is the formation of an ion pore/channel in response to an associating stimulus. Montal and Borochov-Neori (University of California)

examined whether vertebrate rhodopsin mediates changes in membrane ion permeability by aggregation following the light-dependent conformational change of the molecule. Conductance characteristics of planar bilayers containing rhodopsin support this view of channel formation. However, the alteration in aggregation state after bleaching (determined by fluorescence-energy transfer between rhodopsin monomers) is more subtle than expected.

Cast in the role of principal information carrier associated with the operation of membrane channels, ionic calcium continues to attract much attention. The eager studies of the action of intracellular calcium have often overshadowed the more sober, but hardly less important, investigation of its intracellular regulation. Although there is still confusion and controversy about how calcium enters the cytoplasm, steady progress has taken place in measuring it when it is there and on how it is removed to return the cell to a resting state. For example, using permeant fluorescent calcium indicators synthesized by R.Y. Tsien (University of Cambridge), resting lymphocyte calcium ion was measured at 0.12 µM.

For the extrusion of calcium ions, two plasma-membrane processes are available: a Na<sup>+</sup>/Ca<sup>2+</sup> exchange protein and a

calcium-stimulated ATPase. Blaustein and Nelson (University of Maryland), DiPoldo and Beauge (Caracas and Cordoba, Argentina) and Caroni and Carafoli (University of Zurich) all described a calcium removal process operating at very low levels of Ca<sup>2+</sup> (down to 0.1μM), fueled by ATP. However at high Ca<sup>2+</sup> (up to 10 μM), Na<sup>+</sup>/Ca<sup>2+</sup> exchange was dominant. The latter authors reported that the cardiac muscle sarcolemma Na<sup>+</sup>/Ca<sup>2+</sup> exchanger swops 3Na<sup>+</sup> for 1 Ca<sup>2+</sup> (requiring a counterion) and is insensitive to calmodulin, unlike the Ca<sup>2+</sup>-ATPase.

High intracellular Ca2+ was formerly thought to regulate the permeability of the intercellular gap junction. As related by M.V.L. Bennett (State University of New York), the association constant for this process is unphysiologically high (K =0.4mM Ca2+). In single pairs of embryo blastomeres connected by gap junctions, conductance is regulated by a process with a  $pK_a$  of 7.3 (the channels are closed when the cytoplasm is slightly acidic). Voltage clamping the cells at different levels, thus forcing a transjunctional potential, also closes the channels. These two control processes are not the same, since aldehydes selectively block the pH dependence.

Work on the mammalian liver cell gap junction has also progressed well; a hexameric array of protein units spans the

#### 100 years ago

#### POPULAR NATURAL HISTORY

We give, through the courtesy of the publishers, another illustration taken from the chapter on Weevils. It is of a weevil known as Rhyncophorus palmarum. Its fat grubs live on the stems of palmtrees, and are often very destructive. Several of the species are very injurious to the sugar-cane. One found in sugar-plantations in Guiana contain in their intestines lumps of a sweet waxy substance - the altered saccharine food on which they live - and for this they are boiled and eaten by the native. The fine fat larva and the pupal condition, as well as the full-grown weevil, are to be seen in the engraving.

From *Nature* **25**, 109, December 1, 1881.





membranes of the communicating cells. B. Zampighi (UCLA) described a new iunction for eye lens fibre membranes. At low resolution, the protein subunits are tetramerically arranged, unlike the conventional gap junction.

Many functions of intrinsic membrane proteins depend on ATP, and in the last

session delegates were rewarded with the sight of NMR spectrometers built to measure ATP in most parts of Britton Chance's anatomy. The impact of this lecture was certainly magnetic and the closing slides of the ultimate biophysical conjunction, man and machine, were a fitting way to end the Congress.

# Control of a mixed tRNAprotein operon

from Andrew Travers

DURING exponential growth the bacterium Escherichia coli balances the production of ribosomes and other necessary components of the translation machinery of the cell so that no more ribosomes are produced than are required for the maintenance of growth. To achieve this balancing act the synthesis of the stable RNA species, rRNA and tRNA, must be accurately coordinated with the synthesis of both ribosomal proteins and the cycling translation factors. One such protein is the elongation factor Tu (EF-Tu) whose function is to bind charged tRNA and facilitate its engagement in the acceptor site on the ribosome as a prelude to peptide bond formation. Accordingly, the amount of EF-Tu in the cell parallels that of tRNA. The protein is one of the most abundant in E. coli comprising up to five per cent of the total cellular protein and is encoded by two distinct genes, tufA and tufB, whose products differ by only a single amino acid1,2.

The major gene for EF-Tu, tufA, is expressed at  $\sim 2.5$  times the rate of tufB, and is located in the centre of a cluster of r-protein genes with which it is cotranscribed. This arrangement is typical of r-protein genes in general. By contrast, recent work by three groups3-5 has demonstrated that tufB is not associated with other r-protein genes but instead is cotranscribed with a neighbouring group of four tRNA genes, thrU, tyrU, glyT and thrT. This transcription is directed by a promoter lying upstream of the tRNA genes and is terminated immediately distal to the tufB gene. Although cotranscription of tRNA and protein genes has previously been observed in mitochondria this is the first report of this type of gene organisation on the bacterial chromosome. Moreover Hudson et al. (see this issue of Nature p.422) suggest that this example is not unique and that the tyrT transcript may encode both two tRNA<sub>1</sub>Tyr sequences and a small arginine rich protein. In this case, however, the evidence for the production of such a protein in vitro remains to be established.

What is the functional significance of the

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co-transcription of stable RNA and r-protein genes? Hudson et al. advance two possibilities. The presence of a tRNA structure immediately preceding the tufB coding sequence could enhance the stability of the tufB mRNA. For this hypothesis the in vivo evidence offers as yet little support. Alternatively, the cotranscription of tRNA and mRNA would result in a measure of coordinate regulation. The transcription of both stable RNA and r-protein mRNA species is regulated both by the availability of amino acids and by variation in growth rate. In vivo, sudden amino acid depletion results in a drastic curtailment of the production of all major components of the translation machinery. This stringent control is mediated largely at the level of RNA chain initiation and is associated with the presence of a G+Crich discriminator sequence located close to the start point of transcription.

In addition, promoters regulated in this mode often, but not invariably, contain extensive regions of dyad symmetry. The promoter for the tRNA - tufB transscription unit contains both these features and is indeed controlled stringently both in vivo6 and in vitro4.

For control by amino acid availability, the co-transcription of stable RNA and an r-protein mRNA is logical. However, a paradox is apparent when the linkage of transcript production to growth rate is considered. As the growth rate of a bacterial culture increases there is a proportionate increase in the number of ribosomes per unit amount of total cellular protein. Since the ratio of production of total protein increases in direct proportion to the growth rate (µ) the synthesis rate of both stable RNA and r-protein must increase approximately in proportion to  $\mu^2$  to yield a preferential enrichment of ribosomes. However, whereas the transcription of both rRNA and the most abundant tRNA species follows this latter pattern that of r-protein mRNA does not, increasing only in direct proportion to  $\mu$  (ref.7). Thus the growth rate dependent regulation of r-protein synthesis is probably mediated by post-transcriptional controls, including the feedback inhibition of r-protein mRNA translation by free r-proteins. By fusing

rRNA and r-protein promoters to the coding sequence for galactokinase or B-galactosidase Miura et al. 8 have recently demonstrated that the growth rate dependent regulation of transcription is programmed by the promoter region and thus probably reflects variation in rate of RNA chain initiation.

This pattern of differential initiation at stable RNA and r-protein promoters raises the question of whether the growth rate regulation of transcription of the mixed tRNA-tufB operon resembles that of r-protein mRNA or of stable RNA. Whatever the answer it is apparent that regulation of at least one portion of the operon would be anomalous.

No direct evidence bears on the question nor does the tufB promoter sequence offer any obvious clues. However, the function of the tRNA products of the operons favours the possibility that the whole operon is regulated in the r-protein mode. At high growth rates the r-proteins comprise the major class of proteins synthesised in the bacterial cell. Their translation, as well as that of EF-Tu itself, 1.2 preferentially utilises a limited subset of possible codons. Thus those tRNA species which recognise the codons included in this subset will be required in high amounts at high growth rates. Of the tRNA species contained within the hybrid operon two, the products of thrU and glyT. do not utilize the preferred codons. A third, the product of thrT, does recognise codons in this class but is the minor species of the two tRNAThr isoacceptors which do so. Thus there is no obvious requirement for the products of these three tRNA genes to be preferentially synthesised at high growth rates. However, the product of the fourth tRNA gene, tyrU also utilizes a preferred codon and is 1.5 times as abundant as the product of the second tRNATyr gene, tyrT. Nevertheless, the tyrT gene contains two copies of the tRNA sequence in a single transcription unit, an arrangement similar to that for the major tRNAGly, a tRNA utilized preferentially at high growth rates. Thus with the possible exception of the tyrU product all the products of the mixed operon, both tRNA and protein are minor components of mixtures of molecules of similar function. Such a situation would be more compatible with the pattern of r-protein regulation than with that of stable RNA regulation. In accord with this possibility the production of the tufB is less sensitive to amino acid depletion than is the tufA product6 and so would be favoured under conditions of growth rate limitation imposed by lack of amino acids.

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# The geological exploration of Tibet

from A.M.Celâl Sengör

Owing to its unique physiography, geographical location and culture, the Tibetan Plateau has long attracted international scientific interest. Tibet owes its uniqueness to its very high elevation (~ 5 km above sea level), its vast areal extent ( $\sim 2.5$  million km<sup>2</sup>) and its position in the rain shadow of the Himalayan Range (Fig.1). Tibet's elevation has been attributed to a double thickness (~ 70 km) of continental crust underlying it1, but the precise mechanism for the development of this thick crust and the elevated plateau has been hotly debated. The lack of data from Tibet has been the chief factor in allowing widely divergent opinions to be held on even the most critical scientific questions concerning it.

A number of European and Sino-European scientific expeditions penetrated Tibet during the last decade of the 19th and the first four decades of the 20th century and gathered a large amount of data, pertaining to various disciplines, before such expeditions became impossible owing to the political changes in the area. The great scientific value of Tibet is appreciated by the Peoples' Republic of China, as evidenced by the number of diverse Chinese expeditions devoted to the exploration of Tibet since 1951. From 25th May to 14 June 14th, 1980, Academia Sinica organized a multidisciplinary, international symposium, including a field excursion on the Tibetan Plateau, both to report the results of the three-decade-long Chinese studies to the international scientific forum and to exchange opinions with their foreign colleagues<sup>2,3</sup>. The symposium ended with mutual wishes for future scientific collaboration.

The three papers in this issue of *Nature* (see p.405–417) by a Franco-Chinese team report the preliminary results of a three-month field trip in Tibet in the summer of 1980 and represent the first fruits of the renewed cooperative efforts of Chinese and foreign scientists in Tibet. Because so few foreign scientists have been to Tibet, let alone worked in it, the Franco-Chinese cooperation and the publication of its initial results in a language easily accessible to foreigners is of great importance.

The problems that most students of Tibetan geology are currently trying to address can be divided into two major groups. (1) Although there is general agreement that the basement of the Tibetan

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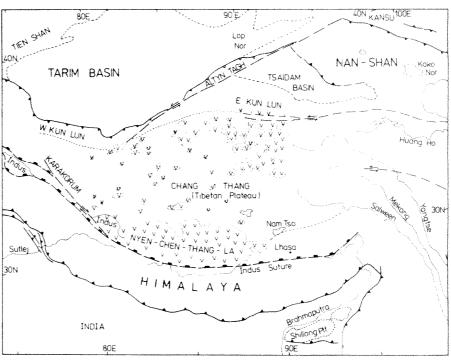


Fig. 1 Tectonic sketch map of the Tibetan Plateau and surrounding areas. Lines with black teeth are active thrusts around the plateau, lines with open teeth are inactive thrust boundary. v = Neogene and younger volcanic rocks (from ref. 11).

Plateau is composed of a small number (two?) of blocks that detached from the northern periphery of Gondwanaland and subsequently accreted to Asia, the exact history of these events is still a matter of conjecture. The elucidation of this history and the sequence of events that accompanied the India-Eurasia convergence, and their precise timing, constitute what I call the first-order palaeotectonic problems of Tibetan geology. (2) It is clear that the evolution of the present Tibetan high plateau is somehow related to India Eurasia collision during the early Tertiary1, but exactly how has been and still remains the question. This seems to be the first-order neotectonic problem of Tibetan geology. The three papers in this issue deal with both groups of problems in their study areas and also make some generalizations that pertain to the entire plateau.

Tapponnier et al. (p.405) review the background and general results of the cooperative field-work in southeastern Tibet. Their stratigraphical observations on the Lhasa Block are consistent with the earlier inference that this block, bounded in the north by the Tanggula suture of late Jurassic-early Cretaceous age and in the south by the Yarlung-Zangbo suture of early Tertiary age, was, until the Triassic, a part of Gondwanaland. The most

interesting novelties reported are those that pertain to the evolution of the Yarlung-Zangbo suture, the lineament that marks the line of apposition between the Indian sub-continent and Eurasia following the late Cretaceous-early Tertiary demise of Neo-Tethys. Tapponnier et al. argue that the structural evolution of the Yarlung-Zangbo suture involved four distinct episodes of deformation in the region they studied. Although the precise timing of these episodes and that of the emplacement of the Yarlung-Zangbo ophiolites remains controversial, the kinematic sequence they describe is a considerable advance. Their discovery of the apparent continuity between the epi-ophiolitic pelagic sediments and the unconformable clastic cover of the Gangdise granodiorites strongly supports the earlier interpretations of the Xigatse basin as a fore-arc basin<sup>2,4</sup> and indicates that it probably had an ophiolitic basement. They also underline the importance of the distinction between the Triassic passive continental rise approns2 (under the unfortunate designation "Triassic Flysch") and the Cretaceous wildflysch linked to orogenic deformation. Although there is sufficient evidence to the contrary, Tapponnier et al. reiterate the peculiar view that the Gangdise Andean-type arc might equally well have been an island arc!

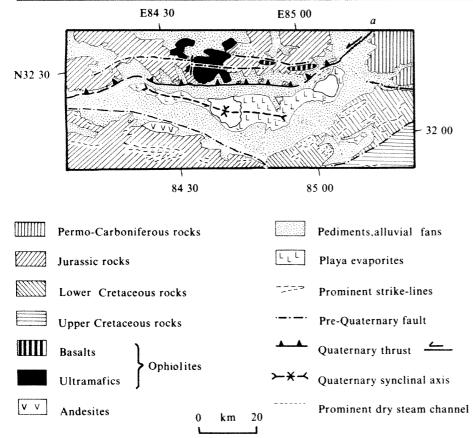


Fig. 2 1:1,000,000 geological map of the Zhaxi Co area drawn from Landsat image no. E-2664-04045-6 01 and sheet 6 of ref.15. White areas are present lakes. a is the strike-slip fault that seemingly connects with the Zhaxi Co thrust, the north-east continuation of which is particularly visible on Landsat image no. E-2663-03584-6 02.

The ophiolites of the suture zone themselves, as exemplified by the Xigatse ophiolite massifs, are interpreted to be unusual examples in the paper by Nicolas et al. (see p.414 of this issue). They note the greatly reduced thickness of the mafic part of the sequence, which is almost devoid of cumulate gabbros. The serpentinized ultramafic part is invaded by numerous diabase sills after the onset of serpentinization. Nicolas et al. prefer to interpret the Xigatse ophiolite as a possible product of a very slowly spreading ridge, although some of the reported peculiarities of this ophiolite complex, particularly the thin mafic section and the late intrusion of diabase into the ultramafic foundation invite suspicion in terms of a near-fracture zone ophiolite<sup>5,6</sup>.

Finally, both papers by Tapponnier et al. emphasize the east-west extensional nature of the Quaternary and also possibly late Tertiary deformation both in the region visited in particular and in Tibet in general, in full agreement with the long declared view of some of the French members of the team<sup>7,8</sup>. They note the decreasing intensity of north-south compressional deformation in southern Tibet since the late Cretaceous and underline the total absence of any Quaternary compressional features such as thrusts or folds in the areas studied. The significance of these preliminary observations increases by the fact that they seem to contradict the views of some Chinese9 and foreign10,11 workers who argue for the existence of Quaternary north-south shortening in Tibet. In fact the most recent geological map of Tibet12 shows a number of east-west striking faults cutting Quaternary deposits in southern Tibet and a few folded areas of Quaternary limnic and fluvial sediments. Figure 2 shows perhaps the most convincing case for Quaternary (active ?) thrusting in Tibet, in the area of Zhaxi Co. On sheet 6 of the 1:1,5 million geological map of Tibet<sup>12</sup>, Zhaxi Co is shown to have perched in a Quaternary syncline. On Landsat images the Zhaxi Co depression is seen to be overriden from the north along a northconcave thrust, which, towards the east, seems to connect with a very prominent east-north-east-striking, left-lateral strikeslip fault (a in Fig. 2) with several sag ponds, dry stream channels bent in an anticlockwise fashion and a very pronounced rift topography. All these structures are very young, if not active. The Zhaxi Co thrust may possibly continue westwards, in the direction of Gerze. Towards the east it is on strike with Siling Co, which is a Neogene (and younger?) ramp basin (Chen Zhi-ming, personal communication, 1980), and with the 1972. 7. 22. earthquake (epicentre 31.4 °N, 91.5 °E) which occurred on an east-west thrust13. All of these very young thrusts seem to be aligned along the trace of the Tanggula suture and

may represent its local, young reactivation. All such observations clearly indicate that the statement "in the Quaternary, deformation of Tibet was characterized by east-west extension" (Tapponnier et al. p.405) may not be descriptive of the entire picture and the comparison of Tibet with such dominantly extensional regions as the Basin and Range and the Aegean, both characterized by thinned crust, may not be exactly appropriate.

Tapponnier et al. also emphasize the "comparatively small amount of shortening and deformation of the surface sediments" north of the Yarlung-Zangbo suture. It would be of great interest to see the actual data and their quantitative results regarding local, observed values of stratal shortening. Because compressive deformation that results in buckle folding is often accompanied by about 10 per cent bulk shortening and thickening before folding occurs, such observations on folding alone always render a minimum value for shortening.

In conclusion, one cannot overemphasize the significance of the recent resumption of Chinese and foreign cooperation in the geological exploration of Tibet. As the papers in this issue and this commentary clearly show, Tibet has a multitude of unsolved yet critical geological problems, the better understanding of which would not only further our knowledge of the regional geology and evolution of this unique structure but would also contribute greatly to our understanding of such fundamental geological problems as continental collision, behaviour of the continental lithosphere, differentiation of the continental crust, and a host of other, smaller ones. No amount of experience and expertise could be too much to bear on such gigantic and diverse problems and if only on these grounds alone the work of the Franco-Chinese team is to be applauded. One hopes it will continue, and other similar efforts be made in the future, to illuminate both the palaeo-and neotectonic problems of Tibet.

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Map of Qinghai-Xizang, scale 1:1,500,000 (Chengdu,

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#### ARTICLES

## The Tibetan side of the India-Eurasia collision

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Results are reported from the 1980 joint French-Chinese field expedition in Tibet. The area covered was from the High Himalaya in the south, to the region of Nagqu ~250 km north of Yangbajain. Ophiolites in the Zangbo valley represent remnants of the crust of an ocean basin which lay adjacent to the Gangdise granodiorite belt in the late Mesozoic. The ophiolites were thrust to the south onto the Cretaceous mélange and Triassic flysch but the age of this event is unclear. In the Tertiary, sediments of the Indian margin and the Xigaze basin were folded with steeply dipping cleavage, which indicates some north-south shortening of the crust. However, the Tertiary volcanic sequence on the Lhasa block and further north shows comparatively little folding and thrusting. In the Quaternary, deformation of Tibet was characterized by east-west extension.

TIBET has been much studied by geoscientists of the People's Republic of China, particularly in the past decade. Important results of these studies have been presented in two recent symposia. These results<sup>1-7</sup> and new maps at a scale of 1/1,500,000 (produced by the Ministry of Geology) provide a good geological data base for a country which is otherwise largely unknown in the west.

The French-Chinese joint study of the 'geological structure, formation and evolution of the Earth crust and upper mantle of the Himalayas' which began with three months of field work in the summer of 1980 provided the first opportunity for western scientists to work in Tibet since explorations at the beginning of this century<sup>8-12</sup>. Hence the results presented here are merely an extension of the Chinese work (see, for example, refs 13-15).

We summarize here preliminary field reports jointly prepared by French and Chinese geoscientists. More detailed accounts will be published elsewhere 16-20. The joint study was limited to a region north of the Himalayas between Zham and Lhozhag, and south-west of Nagqu (~250 km north of Yangbajain). [Note that geographical names are spelled according to the Ping Yin transcription on the most recent maps published in the People's Republic of China: Jiang means river in Chinese and Co, lake in Tibetan.] Some of us (P.T., F.P., X.X.C.) went close to the border with Qinghai, on a reconnaissance trip.

Several important questions about the evolution of Tibet remain essentially unanswered. For example, although there is little doubt that the geology of Tibet was shaped in the Mesozoic by successive collisions with Laurasia of different blocks previously detached from Gondwana<sup>13,21,22</sup>, it is not known precisely how and when the different basins of the Tethys opened and closed. Similarly, although the high and apparently recent elevation of the Tibetan plateau must be related to the collision and penetration of India into Asia<sup>23,24</sup>, detailed field studies of this large and harsh territory are inadequate for a choice to be made between two extreme models—that of Argand<sup>25</sup>, who postulated that Tibet is largely underthrust by the crust of northern India, and that of Dewey and Burke<sup>26</sup>, who suggested

that Tibet has shortened and thickened more homogeneously (like an accordion) during the late Tertiary.

Our main aim was to improve the understanding of the local geology by using new techniques and observations and to integrate our observations into the framework of plate tectonics.

#### Sedimentation changes on the Lhasa block

The stratigraphy of the Lhasa region <sup>1,2,7</sup> has been investigated in particular detail near Linzhu (Fig. 1). North and west of Linzhu, the probably Precambrian metamorphic basement (two mica gneisses north of Machu) is covered by Carboniferous tillites (pass north of Linzhu) with Gondwanian affinities. Shallow marine sedimentation dominates from the Permian to the Jurassic: Permian limestones (including reef limestones), intermediate to basic volcanics and pyroclastics (green tuffs and agglomerates), of Lower Middle Triassic age are succeeded by Upper Triassic to Liassic dark-grey limestones with large pelecypods (Megalodontidae).

In the Jurassic, the deposits become more detrital: Upper Jurassic clastic series with coal lenses, clastic formations with large quartz pebbles in the Lower Cretaceous (similar to those in the Himalayas), rhythmic shale-limestone sequence of Upper Aptian age (Orbitolina sp.). On top of this sequence, red sandstones and siltstones (Takena formation<sup>1,2</sup>), intercalated with calc-alkaline volcanics and oyster-rich limestone beds, gradually become more and more continental upwards (lacustrine limestone and palaeosols). The Gangdise tonalites or granodiorites (Gangdise plutonic belt) and more recent granite bodies (Lhasa-Yangbajain granites) intrude the folded Mesozoic series which are unconformably overlain by red conglomerates with intercalations of purple volcanic tuffs and thick volcanics (Lingzizong formation 1.2). The volcanics (chiefly ignimbrites with dacites and andesites) cap most of the high mountains around Lhasa and north of the Linzhu basin and are probably of Palaeocene age  $^{1,2,18}$ .

The stratigraphy agrees well with the inference that the Lhasa block and its extensions to the west and east is a microcontinent

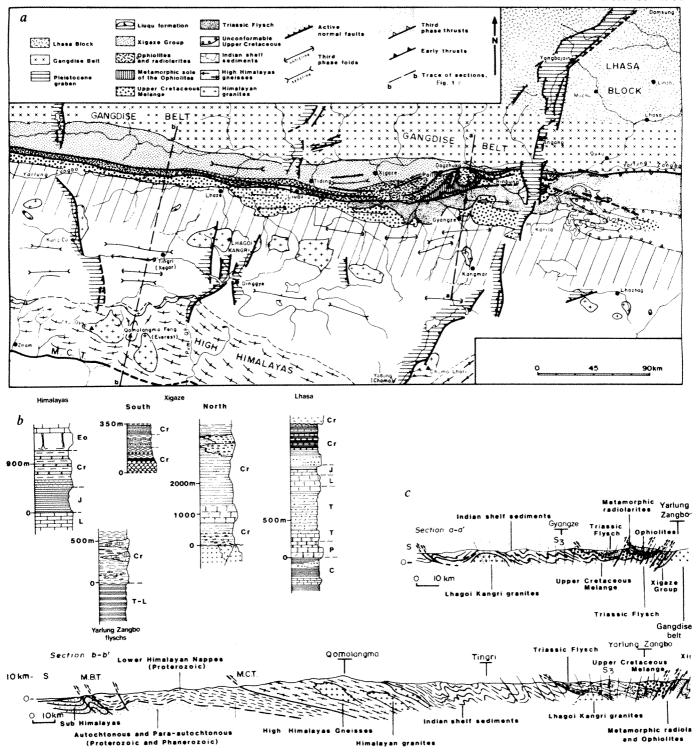


Fig. 1 a, Simplified structural map of southeastern Xizang (Tibet) and Himalayas (after ref. 2). Gangdise belt includes mostly granodiorites and granites but also volcanics (ignimbrites and andesites). Triassic flysch is present in the thinly dotted areas and at some places in the area with oblique bars. Oblique bars refer to sediments of the Indian continental slope which have suffered two phases of deformation and schistosities. The thrust contact between these formations and the more autochthonous Indian shelf sediments (one phase of deformation) is not clear everywhere and has not been represented. b, Stratigraphic columns of the main elements of southern Xizang (Tibet) (this study and ref. 2). Himalayan series (near Tingri): classical succession, from bottom to top: neritic limestones, Lower Jurassic; black shales and marls (Spiti formation), Upper Jurassic; sandstones (Giumal formation), Lower Cretaceous; thin bedded calcschists, with Globotruncanidae, Upper Cretaceous; without any unconformity, thick bedded limestones with abundant microfauna (Nummulites sp.), Tertiary (Palaeocene–Eocene). Zangbo flyschs: base of column is a flyschoid series, pelecypods (Daonella sp., Halobia sp.) give a Middle to Upper Triassic age. Top of column is wildflysch (sedimentary melange), with exotic blocks of various age (Upper Permian, Triassic, Cretaceous), lithology (limestones, cherts and radiolarites, pillow-lavas and so on) and size (cm to km). Globotruncanidae within the matrix give a Campanian (?) Maestrichtian age for this chaotic series which lies in stratigraphic unconformity on top of the Triassic-Jurassic (?) flysch. Xigaze group: south—sedimentary stratigraphic cover of the upper pillow-lavas of the ophiolites: red radiolarites and siliceous shales at the base, microfauna (radiolarians) indicate an Upper Albian, Lower Cenomanian (?) age. Rhythmic flyschoid series follow, with locally interbedded calcareous sandstones and coarse breccias (mass flows reworking lavas and cherts); north—the Xigaze group proper in s

of Gondwanian origin. In the Lower Mesozoic, it was separated from Laurasia by vast ocean basins, whose traces are to be found along the Kun-Lun, Bang Gong-Nu Jiang and other sutures within Central Tibet to the north 13,15. It probably detached from Gondwana in the Triassic or a little earlier<sup>22</sup>. Differences in the sedimentary environment north and south of the Zangbo progressively develop from the Triassic onwards. Until the Jurassic, the shallow marine sedimentary facies are still reminiscent of those on the northern margin of India. In contrast, after the Upper Jurassic, the sedimentary facies are more like those found elsewhere along the northern margin of Neo-Tethys, particularly in the Alpine Middle East. This is additional evidence for a large physical break between the Lhasa platform and the Gondwanian 'mainland' during part of the Upper Mesozoic. The most important tectonic deformations probably took place in the late Cretaceous before deposition of the thick upper volcanics.

# Oceanic crust and sedimentary cover along Yarlung Zangbo

Considerable work by Chinese geologists has drawn attention to the Yarlung Zangbo ophiolites  $^{1.13-15}$ . In the region near Xigaze, the ophiolites are particularly well exposed and form an eastwest belt  $\sim 170$  km long and 2-20 km wide (Fig. 1). Most of this belt was mapped during the summer of 1980 at a scale 1/100,000 and three of the largest massifs (Dagzhuka, Xigaze and Liuqu) have been the subject of particularly detailed structural studies (local maps at a scale of 1/25,000; ref. 16) and geochemical sampling.

On the whole, the ophiolites have not been much dismembered or weathered. Except for conjugate strike-slip faults trending ~N 60 °E and 110 °E within the massifs, the major faults are east-west thrusts of the ophiolites over the sedimentary series to the south. Displacements seem to have been a maximum for the Dagzhuka massif which forms a rather large thrust sheet on a sole of metamorphic rocks. Other massifs such as those near Tiding are made of stacked up thrust slices with some retrocharriage to the north onto the Xigaze group. The Xigaze, Liuqu and Polio massifs have merely been tilted ~60° to the north-west and display a remarkably complete and apparently continuous section. A typical cross-section is that of the Xigaze massif (Fig. 2). From north to south (and top to bottom), the southern rim of the Gangdise granodiorite 'batholith' is unconformably covered by variegated conglomerates and molasses representing locally the base of the Xigaze group. To the south the basal clastic sediments appear to grade into pelagic deposits which form the normal sedimentary cover of the oceanic crust<sup>19</sup>. Typically, a few metres of red radiolarian chert encrust the upper pillow-lavas. Above, the cherts give way to a rhythmic brown-green volcaniclastic formation, 300-400 m thick, with radiolaria-rich horizons at the base, of Upper Albian-Lower Cenomanian age.

The polarity of individual pillows (base to the south-east) is consistent with this description. The pillow-lavas are sometimes intercalated with lava flows several metres thick. Under the effusive volcanics, a peculiar succession of dolerite sills with some dyke swarms and exceptional screens of isotropic gabbro are observed. The dolerites, which form a real 'sill complex', are in contact with serpentinized dunites and harzburgites underneath. Further down (south), fresh peridotites consist mainly of harzburgites interbedded with Cr diopside-rich harzburgites. A shear zone of tectonized serpentine containing ophiolitic blocks, marks the basal thrust of the ophiolite complex on the red radiolarian cherts and coarse chert conglomerates.

If not disrupted by internal low angle shears that could have escaped our attention, the 'Xigaze' ophiolites represent a remarkable type of oceanic crust: the crust must have been thinner (at most 3.5 km thick) than the 'normal' crust (~5 km thick) found in many ophiolite complexes around the world. Also, whereas cumulate gabbros are abundant in typical ophiolites, they appear to be almost absent in the Xigaze complex (only small bodies, probably corresponding to magma chambers, have

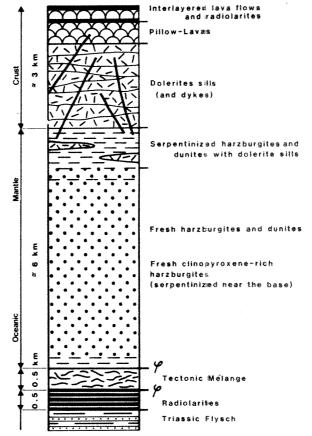


Fig. 2 Simplified section in the ophiolites of the Yarlung Zangbo Suture.

(For more details see accompanying article <sup>32</sup>.)

been observed in the vicinity of Dagzhuka and Tiding<sup>16</sup>). Finally the existence of Cr diopside-rich harzburgites at shallow depths under the crust is unusual: the ophiolitic oceanic mantle is normally composed of residual harzburgites and the occurrence of lherzolites is rare.

An important discovery is the apparent continuity between the normal pelagic cover of the ophiolites and the more clastic sediments which unconformably overlie the Gangdise granodiorites. This implies that the ophiolites of the Yarlung Zangbo suture zone correspond to the oceanic crust of a rather deep basin (the Xigaze basin), located south of the Trans-Himalayan region but at a short distance from it. Although the age of this basin is not accurately known, the radiolarian cherts above the pillows indicate that it must have formed before the Upper Albian. The main molassic units of the Xigaze group seem to have accumulated mostly during the Upper Aptian<sup>1,2</sup> but perhaps until the Upper Cretaceous and Lower Tertiary.

#### Triassic flysch and Cretaceous wildflysch

Between Lhozag in the east and Tingri to the west (Fig. 1), the Mesozoic sediments deposited on the north Indian margin are identical to those known as the 'Himalayan' series farther west<sup>27,28</sup> (Fig. 1): black shales ('Spiti') with nodules, of Upper Jurassic and Lower Cretaceous age, Lower Cretaceous sandstones and quartz pebbles conglomerates, Upper Cretaceous white limestones and mudstones with Globotruncana, and nummulitic limestones (Middle-Upper Eocene). There is no unconformity between the Upper Cretaceous and the Tertiary, which are merely separated by a hard ground in the mountains west of Tingri. Between the thick and monotonous deposits of the Indian margin and the ophiolites of the Zangbo suture, two distinct flysch formations of different ages and nature must be separated: the Triassic flysch and the Upper Cretaceous wildflysch or mélange.

The Daonella-bearing flysch is most likely to be of Triassic to Liassic age and slightly metamorphic (sericite). Facies of the rhythmic sediments appear to be quite similar to those of the Lamayuru flysch recognized in Ladakh<sup>22,27,28</sup>. On the other

hand, the mélange formation, which was long assimilated to the Triassic flysch, is not metamorphic and is best described as a dissociated wildflysch with exotic blocks. The blocks are of various sizes (from a few centimetres to a few kilometres), ages and facies and are sometimes more deformed than the matrix.

The most common elements are: Permian (Djulfian) whitegrey limestones with crinoids and foraminifera (Agathammina pusilla Geinitz, Geinitzina postcarbonica Spandel, Calcitornella heathi Cushman and Waters, Stipulina sp. Lys), Lower Triassic pink limestones (Meekoceras sp.) of 'Himalayan facies', Lower Jurassic (?) 'filament' limestones, Campanian-Maastrichtian limestones (Globotruncana), blocks made of a primary association of pillow-lavas and red or green micrites, grey, red or green cherts and grey chert breccia. Some blocks are composite: fragments of Permian, Triassic and Jurassic rocks are cemented by micritic limestones of Campanian-Maastrichtian age (Globotruncana stuarti or arca (Fig. 3). The largest (kilometre size) olistolithes are primarily formed of fossiliferous Permian limestones and marl limestone rhythmites or pelagic unmetamorphosed Triassic-Liassic flysch. The matrix is a rhythmic sediment with Globotruncana-bearing carbonate beds.

The wildflysch or mélange thus should be at least of uppermost Cretaceous age. Whereas the Triassic flysch probably corresponds to the accumulation prism along the passive northern margin of the Indian continent, the Cretaceous wildflysch undoubtedly formed in a more active tectonic environment, perhaps in the accretion prism of the subduction zone along the active margin of Asia.

# Overthrusting and shortening north of the Himalayas

In many places, the ophiolite belt along the Yarlung Zangbo seems to be limited to the north and south by steeply dipping faults. More detailed observations show that these faults correspond to rather minor and late tectonic events. The most important deformations and displacements have occurred during earlier phases responsible for the large scale overthrusting of the ophiolites to the south. Microtectonic studies permit the identification of four principal phases of shortening, the first and the third being the major ones.

The most intense deformations seem to have taken place during the first phase  $(\varphi_1)$ . They are best observed in the metamorphic sole under the ophiolites. Both the radiolarian cherts and associated pelagic limestones (marbles) and the schists with thin chert, sandstone and metatuff beds underneath show subhorizontal cleavage and a strong stretching lineation striking N 150° to N 180 °E. As the cleavage is parallel to the basal thrust, and the lineation parallel to the apparent direction of transport, the deformation and metamorphism probably occurred during overthrusting. The Triassic flysch is also affected by an early deformation phase with flattish flow cleavage and roughly north-south stretching lineation.



Fig. 3 Close-up of a Triassic block included in a primary Upper Cretaceous mudstone matrix; the whole is reworked as an exotic block in the late Cretaceous wildflysch (~20 km south of Xigaze).

Kilometric size folds overturned to the south or west can be related to this first phase.

The second tectonic phase  $(\varphi_2)$  is still characterized by north dipping thrusts. However, there seems to be no penetrative deformation and no metamorphism associated with this phase. The final effect is an exaggeration of the early overthrusts which brings the ophiolites, the metamorphic cherts and the Triassic flyschs onto the mélange and sometimes the autochthonous series of the continental slope of India.

The pronounced south vergence of the earliest tectonic events in the Yarlung Zangbo zone implies that some of the oceanic crust of the Tethyan Sea was subducted to the north under the Xigaze basin. The deformed cherts and pelagic slates now incorporated in the metamorphic sole of the ophiolites may represent scraped fragments of the late-Jurassic to mid-Cretaceous cover<sup>7</sup> of this crust. However, because the strong deformation linked with the large scale overthrusts also affects the Triassic flysch and the autochthonous series, and it is logical to relate most of the tectonics to the emplacement (obduction) of the ophiolites onto the northern margin of India.

Along the Yarlung Zangbo zone, the Upper Cretaceous mélange, the Triassic flysch and autochthonous Indian series, and the thrust contacts have been folded again by a third phase  $(\varphi_3)$ characterized by upright folds with undecided vergence and fan-shaped cleavage (S<sub>3</sub>) (Fig. 1). The third phase is especially clear in the region near Rinbung where it has produced retrocharriages: slices of ophiolites, radiolarian cherts and Triassic flysch have been emplaced onto the Xigaze group, along south dipping thrusts outlined by fragments of serpentine. In the series of the Indian margin, south of the granite batholiths of the Lhagoi Kangri range, the third phase is the only one to be observed in the Eocene (near Tingri). The intensity of the deformation and the asymmetry of the folds related to this phase increase towards the south into the lower units of the 'High Himalaya slab'. In the Cambro-Ordovician, the folds are clearly south vergent and the flow cleavage synchronous with metamorphism. Yet further down and south, ductile shear becomes predominant in the augen gneisses injected with tourmaline leucogranite sheets parallel to the foliation. The third phase is also essentially responsible for the deformation of the sediments of the Xigaze group whose uppermost units are perhaps of Eocene age. The style of folding is typical of late phases of rather symmetrical shortening: east-west folds, with steeply dipping cleavage (Fig. 4) are slightly south vergent in most of the basin (subvertical southern limbs). The vergence to the south is most pronounced near the southern edge whereas a north vergence appears locally near the Gangdise batholith

According to some of us (P.M., J.A., J.P.B. and M.B.) three independent sets of observations suggest that the early deformations synchronous with the emplacement of the ultrabasic thrust sheets  $(\varphi_1)$  have occurred before the deposition of the mélange<sup>20</sup>: (1) between Gyangze and Lhaze, the mélange or wildflysch overlies unconformably the Spiti series; (2) whereas two phases of deformation  $(\varphi_1$  and  $\varphi_3)$  are observed in the Spiti sediments, only one phase  $(\varphi_3)$ ? can be recognized in the mélange; (3) elements in the mélange have experienced metamorphic deformation (perhaps  $\varphi_1$ ) before their incorporation in the mélange's matrix which displays only  $\varphi_3$  style deformations.

The most recent structures that can be attributed to north-south shortening in southern Tibet are discrete reverse faults, dipping rather steeply to the north or south. In several places (near Tiding, for example), the faults clearly cut folds of the third phase, and thus belong to a distinct tectonic event, here referred to as phase four  $(\varphi_4)$ . This fourth phase is rather modest and does not manifest itself by distributed folding of the series. It is responsible for the accentuation of the retrocharriage of the ophiolites and cherts slices onto the Xigaze group, which obliterates in most places the root zone of the ultramafic nappes.

In the region of Lhasa, Yangbajain and Linzhu, north of the Gangdise granodiorites, the most prominent folding of the

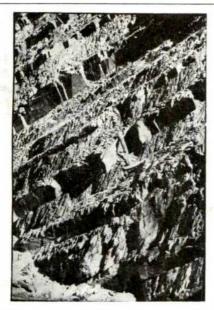


Fig. 4 Well developed steeply dipping slaty cleavage  $S_3$  in the Xigaze rhythmites, 1 km west of Tiding.

Mesozoic sediments, also with steeply dipping cleavage and undecided vergence, has occurred before the Palaeocene: precise  $^{40}$ Ar/ $^{39}$ Ar dating of the upper volcanics (Lingzizong formation) which lie unconformably on the folded continental Cretaceous red beds (Takena formation), but are not much deformed themselves, yielded an age of 60 Myr (ref. 18). The Lingzizong formation is only gently folded and broken by several steeply dipping east—west reverse faults, which can be attributed to the fourth phase of compression identified in the south. Perhaps the most prominent of these faults is that which bounds the Linzhu basin to the north: the fault clearly post-dates the Palaeocene ignimbrites and andesites of the Lingzizong formation which are offset several hundred metres.

Still later in the Tertiary, strike-slip faulting may have occurred, especially in the Zangbo suture zone. We found good microtectonic evidence for right lateral strike-slip faults, striking  $110^{\circ}-120^{\circ}$  N, south of Quxu. There, the suture appears to be more linear and vertical than in the region of Xigaze, a character often indicative of important strike-slip movements. The most recent deformations in Tibet, however, do not correspond to north-south shortening but, perhaps surprisingly, to east-west extension of the crust.

# Tibetan plateau extension during the Quaternary

Although some north-south faults in Tibet had already been described<sup>2,13</sup>, it was the Landsat images<sup>24,29</sup> that first suggested that normal faulting and active east-west extension was a dominant tectonic process over most of the plateau. This inference was supported by fault plane solutions of earthquakes. However, perhaps because extension seemed to contradict prevailing views on how the plateau formed<sup>26</sup>, many have been reluctant to accept it in the absence of more abundant and direct field evidence. We now have an ample collection of field observations<sup>17</sup> which demonstrate that recent distributed normal faulting is as important in southern Tibet as it is in regions such as the Aegean or the Basin and Range. The observations also show how Landsat photos can be used to study active tectonics.

Several normal fault systems cross our study area: from west to east, the most important ones are the Kung Co system, immediately north of Cho Oyu and Qomolangma Feng (Mt Everest) and the Yadong-Karila-Angang-Yangbajain-Damxung-Gulu system, which extends some 600 km from Chomo-Lhari to the south, to the region west of Amdo in the north (Fig. 1) (see also the accompanying paper 30). We were able to map in detail some segments of the Yadong-Gulu graben using aerial photographs 17.

The faults are especially clear in the morphology (Fig. 5). They cut moraines and post-glacial Quaternary terraces in many places (such as Angang, Damxung and Kung Co). In some places glacial valleys have been offset several hundreds of metres, which gives an order of magnitude for cumulated vertical movements since the end of the glaciation. Although most of the faults trend north-south, oblique faults cut or connect them. We found evidence for conjugate strike-slip motion along them.

Five clusters of recent surface breaks have been found west and north of Yangbajain. Uprooted bushes and disrupted turf suggest that most of these have been caused by earthquakes that occurred in the past few decades. The most ancient ones cannot be much older than a couple of centuries. All the breaks reactivate faults clearly visible on the satellite images. Particularly recent breaks are those which run along the western fault of the Gulu graben, south of Gulu, with  $\sim 3$  m of normal slip, and that found south of Lake Peng Co, with  $\sim 5$  m of right lateral strike-slip movement which we relate to the large earthquake of 18 November 1951 (M=8) (see accompanying paper  $^{30}$ ).

Many microtectonic measurements were taken in different favourable sites, either in 'basement' rocks near the master faults (Angang, Damxung and Kung Co) or within the Quaternary gravel beds of terraces (Yangbajain, Angang). Although the data are still being processed, preliminary results are consistent with a roughly east—west minimum principal stress  $(\sigma_3)$  and a vertical maximum principal stress  $(\sigma_1)$ . This stress state should rule out Quaternary folding or thrust faulting, for which we have found no field evidence.

Although no Quaternary volcanics were observed in the region we surveyed, hot springs were numerous and widespread. The rise of hot waters through the crust seems to be a direct consequence of east-west extension because the water often finds access to the surface through north-south cracks. This was particularly clear south of Kung Co, where travertine fissure systems have been mapped in detail.

#### Conclusion

The present field work has improved our knowledge of the geology and tectonic history of southern Tibet. Although it substantiates earlier conclusions 1,2,7,13-15,21,24, it also raises important questions and helps to define priorities for future field research.

It is now established that the Zangbo ophiolites are structurally attached to the Lhasa block and Gangdise calc-alkaline plutonic belt. This supports the idea that the Gangdise belt is the product of northwards subduction of the floor of Neotethys and that the Xigaze basin might have been a fore-arc basin? It is also likely that the 'Lhasa block' is a fragment of Gondwana, but when it became attached to Asia is unknown. For example, we do not know whether the Gangdise batholith was emplaced along an Andean margin, or along an island arc containing some continental fragments. Although great changes can occur along strike, the Ladakh-Kohistan batholith, which is the analogue of

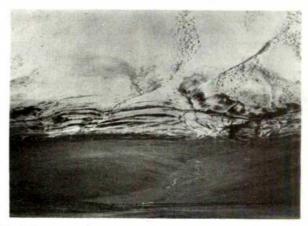


Fig. 5 Spectacular Quaternary scarps, outlined by snow, along the western master fault of the Gulu Graben (north-south trending faults).

the Gangdise batholith in the western Himalayas, has been interpreted as such an island arc in the Mesozoic<sup>3</sup>

The timing of tectonic deformations also raises problems. For instance, the age of emplacement of the Zangbo ophiolites onto the northern margin of the Indian continent is not yet well established. Some of us think it might have occurred earlier than at the end of the Eocene, which would place new constraints on speculations based on plate tectonic reconstructions.

Perhaps one of the most surprising observations was the comparatively small amount of shortening and deformation of the 'surface' sediments, north of the Zangbo ophiolites. Upright folds with steep cleavage predominate and no large-scale tangential tectonics seems to have occurred in the Upper Mesozoic series. Folding and deformation has been especially mila in the Tertiary series around Lhasa and farther north. This is in sharp contrast to the huge thrusts in the lower Himalayas,

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or even typical amounts of shortening in other large orogenic belts and may be related to the flat topography of the Tibetan plateau over most of its surface. If such modest shortening in the Tertiary also prevails far north and west of the regions described here, India's northward push may have elevated Tibet in a different way than accordion shortening and thickening of the crust<sup>26</sup>

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# Field evidence for active normal faulting in Tibet

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Crustal thickening does not now occur in southern Tibet. Field observations made during the Chinese-French expedition of 1980 support an earlier hypothesis based on a combined analysis of satellite images and fault plane solutions of earthquakes. Immediately north of the highest peaks of the Himalayas, the tectonic regime is dominated by east-west extension. We have mapped a large number of north-south normal faults which sharply cut the glacial and post-glacial morphology, and followed several recent earthquake breaks along faults detected on satellite and aerial photographs. Microtectonic measurements in the Quaternary suggest that the maximum principal stress is vertical.

TECTONIC extension sometimes occurs far within continental regions, with no obvious connection with the world rift system and thus the causes are poorly understood. Of all these regions, Tibet is perhaps the most surprising. Since Argand presented his ideas on the tectonics of Asia<sup>1</sup> Tibet has emerged as a symbol of continental collision. Today, within the framework of plate tectonics, there is little doubt that the massive plateau, which maintains an elevation of some 4 km, over 1,000 km north of the Himalayas, is anything but a direct consequence of the most spectacular collision in the Cenozoic-that of India with Eurasia. However, the forces that have pushed up this immense region and somehow thickened its continental crust<sup>1-3</sup> do not thicken it appreciably now. Instead, the crust of Tibet is stretched in an east-west direction. The first field observations of the 1980 French-Chinese expedition firmly establish predictions made with satellite images and fault plane solutions of earthquakes4,5 and substantiate descriptions already made by Chinese geologists both from aerial photographs and in the field<sup>6</sup>. Here we summarize the most spectacular evidence for recent normal faulting and the ensuing preliminary conclusions.

#### Large normal fault systems of southeastern Tibet

As can be seen on Fig. 1, large normal faults cut across the middle reaches of the Yarlung Zangbo River. Figure 1 shows a refined version of earlier maps prepared from satellite images only<sup>5</sup>. Because our field survey was not exhaustive, the present document has also been based on a more detailed interpretation of enlarged and/or better quality Landsat images of the region. However, as we found good correspondence between faults previously recognized on the images and Quaternary faults that could actually be observed in the field, we believe that Fig. 1 represents fairly accurately the pattern of active tectonics in southeastern Tibet.

Between 86° and 91°E, the only area within the limits ascribed to the 1980 French-Chinese study, the normal faults group into three major systems, elongated in a roughly northsouth direction.

Along most of the length of the three rift systems one master fault on one side of the flat Quaternary floor is usually more

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Fig. 1 Map of active tectonics and seismicity of southeastern Xizang and surrounding regions. Earthquake epicentres are from the map of epicentres of strong shocks of China<sup>9,10</sup>. Sizes of circles correspond to magnitudes (small,  $6 \le M < 7$ ; intermediate,  $7 \le M < 8$ ; large,  $8 \le M$ ). Fault plane solution is from ref. 5.

prominent than the other. Sometimes, there is only one such fault, as may also be observed in Greece, western Turkey and the Basin and Range. In addition the faults are discontinuous along strike, often forming en échelon arrays. The most prominent of the three systems is located to the east (Figs 1 and 2a). It extends ~600 km from Yadong, at the foot of the Chomo Lhari range, across the Zangbo suture and the Gangdise granodiorites, all the way to the headwaters of the Nu Jiang (Salween river). Because it is close to Lhasa, and along a major communication axis, we studied it more closely than the others, especially in its central part between the Zangbo and Gulu (Fig. 2b). Abrupt elevation differences along the main faults are typically of the order of 2 km. The three highest peaks of the region are located along the uplifted shoulder of the rift: they rise to >7 km (for example, Mt Noijinkangsang, Mt Nyainqentanglha) (Fig. 1). To the west, the Pum Qu and Kung Co Fault systems (Figs 1 and 2c) were less accessible. In particular, it was not possible to reach regions north of the Zangbo, and the Pum Qu faults between Tingri and Dinggye were seen only during a 2-day reconnaissance trip. Nevertheless, preliminary observations confirmed that the faults are active. The two fault systems are quite different: whereas the faults near Kung Co form a long and linear half graben, those that cut the Pum Qu valley make a cluster of more or less parallel breaks whose continuity across the Zangbo suture zone with the faults that extend northwards to Gyaring Co is not well expressed in the topography.

#### Evidence for post-glacial faulting

In many places along all three 'graben' systems, post-glacial faulting offers spectacular sights. The most numerous and clear examples are found along the Yadong-Gulu 'graben'. We observed three types of morphological evidence.

(1) Quaternary slopes with their ridge and gully topography are sharply cut by the faults (Fig. 3a) along most of their lengths, with typical offsets of a few to a few tens of metres.

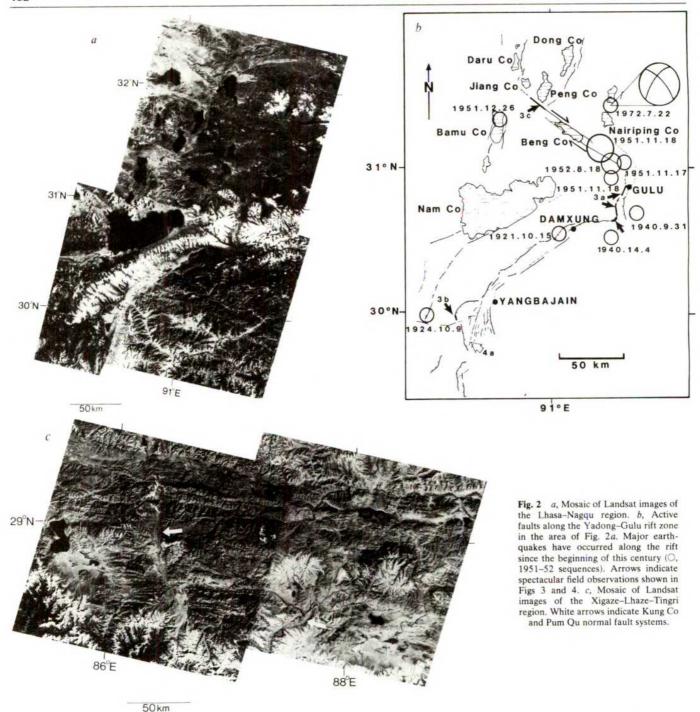
- (2) Near Damxung and Gulu (Fig. 1) attention is caught by several hanging fluvial valleys: ancient valley floors, or flat alluvial fans of tributaries are elevated several metres to a few tens of metres above the present day level of the Lhasa river valley.
- (3) West and north of Yangbajain, the same process, although presumably on a longer time scale, has produced perched glacial valleys (Fig. 3b). This is the most impressive effect of Quaternary normal faulting as valley floors and lateral moraines are sometimes offset as much as several hundred metres.

South-west of Yangbajain (Fig. 1), the Quaternary floor of the Yadong-Gulu 'graben' is cut by a remarkably large number of roughly north-south trending scarps, especially on its eastern side (Figs 1, 2b and 4a). The resulting rift in rift structure, with outward tilt of the Quaternary terraces, is reminiscent of regions of Afar or Iceland, if only because of the fault density. This area will be described in more detail elsewhere<sup>7.8</sup>.

Post-glacial normal faulting is also found along some of the other rifts. For example, spectacularly uplifted glacial valleys, also visible on the satellite images (Fig. 2c), testify for some 500 m of vertical displacement along the master fault near Kung Co, presumably in the past several thousand years. Clearly, more detailed and systematic studies of the post-glacial morphology will be useful for estimating recent extension rates in southern Tibet.

#### Historic surface breaks

Several surface breaks that can be attributed to recent earth-quakes were found along the Yadong-Gulu graben, between the Zangbo and Peng Co (Lake Peng) (Figs 2b, 3a, 3c). All of these reactivate faults that cut the Quaternary morphology, or are located near such faults. Typical vertical offsets are of the order of a few metres. The most recent breaks are observed west of Yangbajain, near Gulu, and near the southern tip of Peng Co: there, the disrupted vegetation has not grown back and the



corresponding earthquakes probably occurred in the past few decades. Epicentres on Figs 1 and 2b are from the historic catalogue of earthquakes compiled by the Institute of Geophysics of the Academia Sinica<sup>9,10</sup>. As for most of Western China, the catalogue contains only shocks which have occurred since the turn of the century, a fact that reflects the low density of population. In addition, we do not know the accuracy with which the epicentres are located, and this makes correlations with surface breaks difficult. Nevertheless, it is tempting to associate the freshest ground breaks near Gulu and Peng Co with the major earthquake sequence that shook the region in 1951–1952.

The sequence, the most important known to have taken place within the Tibetan plateau, was composed of one magnitude 8 shock, one magnitude 7 shock, and at least two shocks with magnitudes >6. All the epicentres cluster near the eastern extremity of a long and linear fault zone (Fig. 2a, b), which we will refer to as the Beng Co fault. This tectonic feature, particularly sharp on the satellite images, has been interpreted as a

predominantly strike-slip fault5,11. One of the major finds of a reconnaissance trip to the region of Peng Co, which was more easily accessible, was a straight alignment of pressure ridges and cracks (mole tracks) in the meadows immediately south of the lake (Fig. 3c). In two places, the rather fresh break, oriented  $\sim N$ 135° E, cut across Quaternary ground morphology and we could estimate a right lateral offset of at least 5 m. This supports the idea that the Beng Co fault is indeed essentially a right lateral strike-slip fault and that it was activated by the magnitude 8 earthquake of 1951. Although the epicentre is located near the eastern extremity of the fault, the break appears to have propagated at least 100 km to the west, to the place where we observed it. The main shock of the 1951-52 sequence would thus be strike-slip but it is not unlikely that foreshocks and aftershocks (Figs 1, 2b) have ruptured the normal faults of the Gulu graben, (Figs 2b, 3a). According to one of us (H.T.), the study of annual growth rings of trees on both sides of the normal fault south of Gulu supports the inference that this fault was activated in 1951-52.



Fig. 3 a, Quaternary normal fault near Gulu. The thin white line (white arrows) which runs along the main scarp probably corresponds to the surface break of one of the 1951–52 earthquakes. b, West of Yangbajain, glacial valley and moraines are cut by a prominent normal fault. c, Eroded mole tracks follow the straight surface break presumed to be that of the magnitude

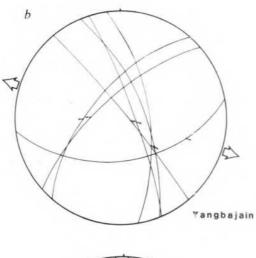
8 earthquake of 18 November 1951.

#### Geometry of faulting and stress in the crust

The observations above (Figs 1, 5) show that the present tectonic regime in southeastern Tibet is dominated by east—west extension. However, as was already suspected from the satellite images, this involves not only north—south trending normal faults, but also strike-slip faults, which sometimes provide the kinematic link between distinct normal fault systems, much as transform faults between ridge segments in the oceans. Such is the case for the Beng Co fault, and probably other faults with similar orientations (for example along Gyaring Co, Fig. 1). Faults with a 'conjugate' strike (N 60° E) also exist and we found good evidence for left lateral movements along them (see Fig. 1, near Yangbajain and Damxung). In addition, the *en échelon* geometry of some normal fault systems (Fig. 1) favour strike-slip components in the deformation of the crust.

These kinematics are born out by our microtectonic measurements either in the Quaternary (Fig. 4a) or in the fractured bedrock and mylonites near the main faults. Although these measurements and the inferences from them will be reported in detail elsewhere<sup>8</sup>, a sample diagram is shown in Fig. 4b. Orientations of the faults are variable, and the corresponding slip on them entails a variable strike-slip component. Nevertheless, all faults have a large normal component and are compatible only with extensional tectonics. Nowhere did we observe reverse faulting or folding in the Quaternary. The extension direction can change from one site to the next, but it is east—west on average. Numerical inversion of the microtectonic data (slickensides)<sup>12</sup> is consistent with a vertical maximum principal stress  $(\sigma_1)$  and a roughly east—west minimum principal stress  $(\sigma_3)$  (ref. 8).





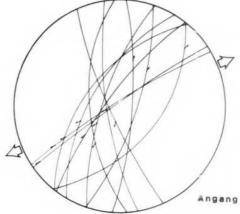


Fig. 4 a, South of Yangbajain: normal fault cutting Quaternary terrace gravels. Many other normal faults occur nearby. b, Measurements of slickensides (arrows) on Quaternary faults (great circles) in the regions of Angang and Yangbajain (lower hemisphere projection) indicate soughly east-west extension.

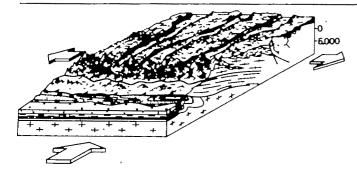


Fig. 5 Idealized block diagram shows how, whereas folding and shortening of the crust still occurs in the Sawaliks and lower Himalayas in response to the northward penetration of India into Asia, east-west extension now predominates on the high Tibetan plateau

#### Conclusions

Although north-south shortening of the crust is evidenced by upright folds and cleavage in early to middle Cretaceous sediments and much more gentle folding in late Cretaceous and early Tertiary sediments 6,13,14, shortening has apparently not been a dominant process in the late Cenozoic tectonics of southern Tibet. During the upper Quaternary at least, but perhaps for a longer period of time, east-west extension must have prevailed on the Tibetan plateau. North-south normal faulting has been especially active in the past several thousand years. As in other areas such as the Aegean in the Mediterranean, or the Basin and Range in North America, such faulting appears to be distributed over a wide area.

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The faults are perpendicular not only to the Himalayan belt in which they stop rather abruptly, but to most of the more ancient Mesozoic and Tertiary fold trends and roughly east-west structural and palaeogeographical zones within the plateau. In particular, the fault systems cut through major sutures such as the Yarlung Zangbo. Thus, although deviation by more ancient structural trends is possible (Fig. 1), a large proportion of the north-south normal faults are probably not reactivated older ones as has often been suggested 15. Hence, perhaps more than in any other region, extension in Tibet seems to reflect a particular state of stress in the crust and upper mantle, where gravity can be the main driving force. Microtectonic observations made during the Chinese-French expedition support this inference.

Although further speculations on the cause of the east-west extension must await a better knowledge of the upper mantle and crustal structure of Tibet, the approximately double thickness of the continental crust as well as the softness of the hot lower crust<sup>16</sup> probably favour east—west flow in response to India's northward push<sup>5,17</sup>.

Now that widespread normal faulting on the Tibetan plateau can be considered more firmly established, important questions remain for future field work: when did incipient extension take over folding and crustal thickening? Does the time when this change occurred compare with the time when Tibet acquired its abnormally high elevation? At what rates has east-west stretching of the crust taken place since then?

New data on these questions will surely place constraints on how large and high plateaus form and evolve during continental collision.

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# The Xigaze ophiolite (Tibet): a peculiar oceanic lithosphere

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The Xigaze ophiolite which outcrops along the Yarlung Zangbo river, southern Tibet, locally displays a complete ophiolitic sequence from marine sedimentary cover over basaltic volcanics to the north, to fresh Cr diopside-rich harzburgites to the south. In contrast with other ophiolites, the mafic part of the sequence is particularly thin. It is almost devoid of cumulate gabbros consisting of a diabase sill complex covered by lava-flows or pillow-lavas. The ultramafic unit is poor in residual harzburgites and dunites and consists dominantly of fresh Cr diopside-rich harzburgites. The origin of this peculiar ophiolite (of oceanic crust) is discussed.

OPHIOLITE complexes in various parts of the world display remarkably comparable sections with, from top to bottom, basaltic lavas, dolerite dykes, isotropic gabbros, cumulate gabbros and ultramafic rocks, resting on tectonic dunites and harzburgites. The mafic part of these complexes usually represents a thickness of between 3 and 6 km (refs 1,2). It is this remarkable homogeneity, at least in their gross features, which led to the concept of ophiolites and to its informal definition3. In contrast to this homogeneity, various oceanic environments have been considered as potential sites for the origin of ophiolites: oceanic ridges with various spreading rates<sup>4-10</sup>, back-arc basins<sup>11,12</sup>, ridges with various spreading rates<sup>4–10</sup>, back-arc basins<sup>11,12</sup>, island arcs<sup>13,14</sup> and even transform faults<sup>15</sup>. The Xigaze ophiolite outcrops in Thet aleast the T. outcrops in Tibet along the Indus-Zangbo suture zone, one of many sutures which separate the Indian continent from the northern plate(s). Only limited data are available about these ophiolites 1,10 although they have been discussed in a recent report16 that draws attention to the presence of limited amounts of cumulate gabbros and suggests that they may well correspond

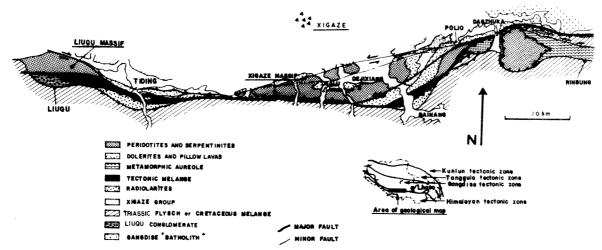


Fig. 1 Geological map of the Yarlung Zangbo ophiolite belt around Xigaze.

to a slow-spreading ridge on the evidence of a "thin, perhaps discontinuous plutonic sequence".

Considering that, as far as we know, ophiolites without cumulate gabbros have been identified only in highly dismembered massifs, the detailed structural study of the Xigaze ophiolite was of particular interest. It should contribute to the discussion on the relation between the existence of magma chambers in the oceanic crust beneath spreading centres and the problems of rate of partial melting in the mantle and rate of oceanic expansion.

#### The Xigaze ophiolite

The Indus-Zangbo suture in Tibet is commonly marked by a discontinuous line of serpentinites or by minor ophiolite bodies. The Xigaze ophiolite belt is >170 km long in the east-west direction of the suture and has a maximum width of 20 km (Fig. 1). It contains three areas where large masses of ultramafic and mafic rocks have been preserved from the intense deformations observed elsewhere along the suture zone: the Dagzhuka, the Xigaze and the Liuqu massifs. In the Dagzhuka and Xigaze massifs, a sequence from sedimentary cover over basaltic volcanics to the north to fresh harzburgites rich in Cr diopside to the south has been identified. Together with more partial sections such as those of Dejixiang, Polio and Tiding they enable a schematic cross-section to be constructed for the Xigaze ophiolite (Fig. 2).

Due to collision along the Indus-Zangbo suture zone, the ophiolites in the Xigaze area have been tilted into a 060°, 60°NW attitude with only local variations. As a result, the top of the sequence and its sedimentary cover are located to the north of the section. Generally, the main lithological contacts in the ophiolite, oriented at 060°, 60°NW, are few tectonized. They contrast with the east-west contacts, the main one being the southern limit of the ophiolite, which cut the former and are systematically tectonic.

From north to south (Fig. 2) one first meets the Gangdise 'batholith' which is commonly ascribed to an island arc or a continental active margin environment<sup>13,18</sup>. Its location relative to the suture, its east—west orientation and its lithologies point to the presence of an underlying, northward subduction zone. The northern part of the Xigaze group is considered as an essentially detrital formation accumulated on the southern margin of the granodiorite (Gangdise) massif. Its contact with the ophiolite often is a stratigraphic one with a few metres of cherts over the basaltic volcanics, covered by marine pelagic sediments reworking in various proportions the underlying volcanics<sup>19</sup>.

Below the cherts either pillow-lavas or lava-flows are observed (Fig. 3). They are parallel to the chert orientation with local departures in the pillow-lavas. The polarity in the pillow-lavas is always with the top northward. Their diameter in section is smaller than 60-80 cm and they are often extended in tubes with no particular directions. They are either largely variolitic,

with 1-cm varioles or massive with only a finer grain rim. Their matrix is not abundant and exclusively constituted by volcanic debris and glass. Beneath a few hundred metres of volcanics, the first diabase dykes and sills appear. They typically present chilled margins and have been observed cutting through the pillows. They progressively become more abundant until they constitute a sill complex with sills intrusive into sills. The sill rather than dyke nature is deduced from their general parallelism with the overlying volcanic lava-flows and sediments. A few dykes, normal to the sills, are often present (Fig. 3); these can be very abundant in some massifs such as Dagzhuka. The thickness of individual sills is in the metre range. When they reach a few metres, microgabbro textures can be obtained in their core. New sills intrusive in such rocks can create the illusion that they are intrusive in a fine-grained gabbroic formation. True plutonic rocks are uncommon between diabase sills with the exception of a few isotropic gabbros and trondjhemite screens.

In the Dagzhuka and Tiding massifs (Fig. 1), small bodies of cumulate gabbros and ultramafic rocks are lecally present at the base of this sill complex. In these massifs, the thickness of the cumulate olivine gabbros is <170 m and <120 m respectively. In the Dagzhuka massif, the walls of the chambers are made of troctolites and dunites which can be intempreted as the first cumulates or, alternatively, as residual wells impregnated by feldspar coming from the chamber. The shape of the magma chambers is very irregular and, in Dagzhuka, the total thickness mentioned above could be achieved by addition of smaller chambers. In Tiding, there is a remarkably thick sequence of isotropic gabbros which may represent the upper part of a comparatively large magma chamber.

The thickness of this crustal unit does not exceed 3 km. It lies over a serpentinized harzburgite and dunite formation penetrated by numerous diabase sills. Those sills which can constitute 50% in volume of this new formation are often particularly thick, up to 7 m. They have been observed to branch to perpendicular dykes which probably represent their feeders. A few rodingitized gabbro dykes also intrude the ultramafic rocks but they are cut by the diabase ones. In contrast with the gabbros, the diabase dykes seem to be usually fresh or rodingitized only along their margins. As rodingitization probably accompanies the serpentinization of the periidotite20, it can be concluded that the gabbro dykes were emplaced before the serpentinization and the diabase ones, after itsor late during the process. The peridotite structure is not altered by the serpentinization except locally where low-temperature deformations have disrupted and boundinaged the dykes and developed a slickenside cleavage in the serpentinites. As a consequence the coarse porphyroclastic texture of the peridotite, typical of hightemperature flow<sup>22</sup> is still visible and its orientation can be mapped. The foliation is represented with its field orientation in Fig. 2 and with its restored orientation in Fig. 3. The thickness of

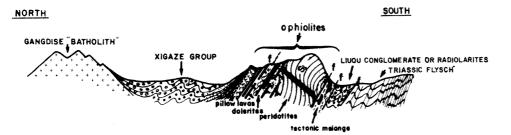


Fig. 2 Schematic north-south crosssection through the Yarlung Zangbo suture near Xigaze. S<sub>1</sub>, foliation.

this harzburgite and dunite formation containing the sills is 500 m on average with important local variations. Downwards it grades into a 500-m unit formed of serpentinized foliated harzburgites and dunites with a decreasing amount of gabbro and diabase dykes. The degree of serpentinization rapidly decreases in these peridotites. After 1-2 km of relatively fresh harzburgites and dunites, fresh harzburgites with a crude layering resulting from the alternation of Cr diopside-rich and Cr diopside-poor facies, visible at various scales are encountered. No banding is observed in these rocks except for a few pyroxenites. The rocks are well foliated with discordant shear zones represented by a few metres of mylonitic peridotites in the Xigaze and Liuqu massifs (Figs 2, 3). Outside these bands, fine to coarse-grained porphyroclastic structures related to hightemperature plastic flow are observed. At the massif scale, the tectonic structures display a relatively irregular pattern though remaining consistent from one massif to another1

The southern east-west contact of the Xigaze ophiolite with the sedimentary formations is tectonic. Over 500-800 m, the ultramafic rocks are transformed into an ophiolitic mélange with blocks of rodingitized gabbros and diabases floating in a schistose serpentinite. On the other side of this major tectonic contact, either radiolarites or a coarse red conglomerate separate the ophiolites from the Triassic flysch series. They are also strongly deformed and schistozed at the contact but, like the serpentinites, are devoid of any metamorphism.

Amphibolites, garnet amphibolites and quartzites have been observed in several localities (Liuqu, Bainang and Dagzhuka) as inclusions in the serpentinite mélange. In the Dagzhuka massif, mylonitic peridotites are developed over several dozen metres above the basal contact which contains blocks of amphibolites in a serpentinite matrix. Beneath, a metamorphic formation consisting of quartzites, phyllites and locally ophicalcites, outcrops over 300 m. This might represent the basal part of the metamorphic aureole well known in many ophiolites<sup>22,23</sup>.

#### Discussion

Considering the general tectonic situation, special attention must be devoted to the question of continuity within the ophiolites mainly at the critical level between the mafic and ultramafic formations. This continuity is thought to be locally preserved for the following reasons.

First, the sills and the dykes are remarkably continuous, keeping the same attitude from the volcanics down to the upper peridotites; and second, the same general sequence is systematically observed along the area studied (Fig. 1). In particular, the possibility of a squeezing out of the gabbros is remote, considering that no large masses of gabbros have been found. On the other hand, isolated pockets of gabbros are now well identified within the mafic part of the sequence. We thus think that the gabbros have not been faulted out and that a continuous section was observed in the Xigaze area.

The specific characters of the Xigaze ophiolite can be summarized as follows:

(1) In contrast with other non-dismembered ophiolites, the mafic part of the sequence is nearly devoid of plutonic rocks except for the small cumulate gabbro bodies found in the Dagzhuka and Tiding massifs. The mafic formation is entirely comprised of basaltic volcanics overlying diabases and dolerites with isotropic gabbro screens and, more rarely, trondjhemite screens.

- (2) The diabase unit is a sill complex and not a dyke complex, as expected, with sills intrusive one into the other and only a few branching dykes at right angles to the sills. This has not yet been described in ophiolites except in the Point Sal ophiolite<sup>24</sup>.
- (3) This mafic part of the sequence is remarkably thin (compared with that measured in non-dismembered ophiolites) with local variations in thickness, but does not exceed 3 km.
- (4) The upper harzburgites and dunites are invaded by thick diabase sills over a thickness of  $\sim 1$  km. To our knowledge, this is also unique as diabase dykes are very scarce in the few ophiolite massifs where they have been described cross-cutting the harzburgites<sup>25</sup>.
- (5) Cr diopside-rich harzburgites are abundant in the ultramafic unit and appear only at 2 km beneath the mafic unit and at 5 km beneath the sedimentary cover of the ophiolite sequence. This is unique as the ultramafic unit in ophiolites is usually composed of depleted harzburgites with  $Cr_2$  diopside-rich harzburgites appearing only in the basal section in a few massifs<sup>27</sup>.
- (6) Local thin shear zones inside the ultramafics with low-temperature plastic flow structures have not been described in ophiolites. Those observed are generally located at the base of the ultramafics. They are thicker and clearly related to late thrusting of the lithosphere<sup>28</sup>.

Depending on the spreading rate, the structural, petrological and geochemical character of oceanic spreading centres are expected to vary. The difference seems to be essentially controlled by the heat budget<sup>29,30</sup>. Slow-spreading ridges will have a lower rate of magma generation and therefore of heat production for a comparable thermal dissipation rate. As a

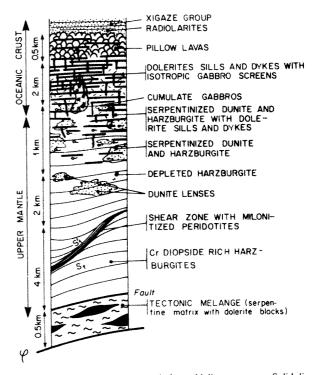


Fig. 3 Schematic cross-section of the ophiolite sequence. Solid lines represent the foliation S<sub>1</sub>. The smaller the spacing, the higher the strain. The various attitudes have been restored after a rotation to horizontal of the radiolarites and basalt flows.

result, magma chambers where the gabbro cumulate sequence crystallizes are not expected for spreading rates of <1 cm yr (refs 31, 32). Steeper isotherms in the mantle should tend to concentrate the magmatic and tectonic activity closer to the ridge than in fast-spreading ridges. However, as mentioned elsewhere<sup>33</sup>, one of the bases for these thermal models is that the upwelling rate of the asthenosphere is proportional to the rate of spreading. The fact that spreading is a discontinuous phenomenon is likely to change these conclusions.

If these conclusions are applied to the Xigaze ophiolite, our evidence indicates a crust formed at a spreading centre with a particularly slow spreading rate, <1 cm yr<sup>-1</sup> if one accepts this limit for the transition from continuous to discontinuous magma chambers. This interpretation differs significantly from that of Wu Haoruo and Deng Wanning14 who think—based on major element chemistry—that the Xigaze ophiolite has been formed in a subduction zone environment. Further detailed geochemical studies are necessary, however, as most of the studied mafic rocks are altered.

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Although the mafic crust in our case is only  $\leq 3$  km on average. the geophysical Moho would be deeper, considering that the 1-2 km of serpentinized peridotites with up to 50% in volume of diabase sills display crustal seismic velocities. Our evidence that serpentinization occurred before the diabase emplacement shows that these peridotites were serpentinized at the ridge itself and therefore that they belong geophysically to the crust. The 'geophysical crust' would therefore have a thickness comparable with that determined by a  $V_p$  transition from  $6.8 \,\mathrm{km \, s^{-1}}$  to 8.1 km s<sup>-1</sup> for slow ridges like the Atlantic one<sup>3</sup>

Finally, if the interpretation that the Xigaze ophiolite originated at a slow ridge is correct, the study of its structure, petrology and geochemistry bears directly on the structure and nature of slow ridges. In this respect, the occurrence of a sill complex as the main component of the crust, which also extends in the uppermost mantle, is interesting.

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# Antibodies to left-handed Z-DNA bind to interband regions of **Drosophila** polytene chromosomes

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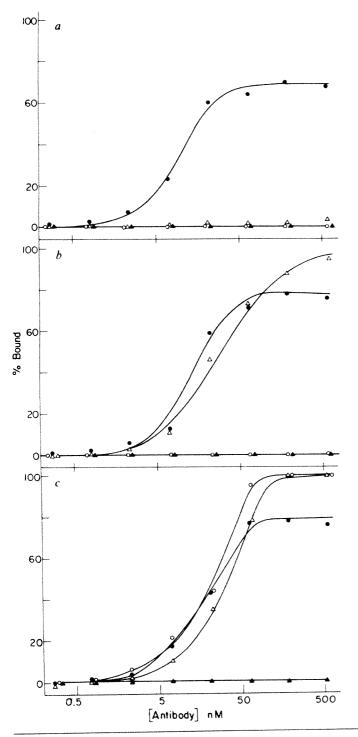
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Antibodies which are specific to the Z-DNA conformation have been purified and characterized on the basis of their binding to three different DNA polymers which can form this left-handed helix. These antibodies bind specifically to polytene chromosomes of Drosophila melanogaster as visualized by fluorescent staining. The staining is found in the interband regions and its intensity varies among different interbands in a reproducible manner. This is the first identification of the Z-DNA conformation in material of biological origin.

LEFT-HANDED double-stranded DNA was first discovered by an atomic resolution X-ray crystallographic analysis of the hexanucleoside pentaphosphate CpGpCpGpCpG (ref. 1). In this conformation the DNA formed a double helix with Watson-Crick base pairs and antiparallel sugar-phosphate chains; the sugar-phosphate backbone followed a zig-zag course and for this reason was named Z-DNA. In Z-DNA the guanine bases have rotated about the glycosydic bond and have assumed a syn conformation, in contrast to the anti conformation in B-DNA. The early observation by Pohl and Jovin<sup>2</sup> of salt-induced inversion of the circular dichroism spectrum of poly(dG-dC)

DNA in 4 M NaCl solution can now be understood as the conformational transition of the right-handed B-DNA to the left-handed Z-DNA<sup>3,4</sup>. Z-DNA has been seen in several crystal structure and fibre analyses<sup>5-8</sup> and generally, DNA with alternating purine-pyrimidine sequences may be expected to have Z-forming potential.

We have recently demonstrated that left-handed Z-DNA is a strong immunogen; in both rabbits and mice antibodies can be raised which are specific for the left-handed Z conformation and will not react with right-handed B-DNA9. Because of their specificity, antibodies can be used to determine the distribution of Z-DNA in biological systems. Here we have purified antibodies to Z-DNA from rabbit sera and demonstrate their specificity for the left-handed Z conformation. We have used these antibodies for indirect immunofluorescent staining of Drosophila melanogaster polytene chromosomes. Because a large polytene chromosome can consist of a thousand or more individual chromatids in very precise alignment, these chromosomes provide a magnification that cannot be obtained with other types of chromosomes. In addition, polytene chromosomes are interphase chromosomes, active in both transcription and replication, in spite of the relatively condensed state of the chromatin. The alignment of the many chromatids making up a polytene chromosome is so precise that regions of tight coiling in the individual chromatids match up to give the appearance of bands across the width of each chromosome. These bands are separated by interbands in which the chromatid fibres are relatively extended. The banding pattern of each



chromosome is essentially constant from nucleus to nucleus and from individual to individual, and must thus reflect basic features of chromatid structure, although the significance of this structure is not understood. Polytene chromosomes therefore provide a unique opportunity for studying the conformational states of specific chromatin regions at times when the chromatin is genetically active.

Indirect immunofluorescence has been widely used to study the distribution of proteins on polytene chromosomes<sup>10</sup>, and here we apply this technique to investigate the occurrence and distribution of Z-DNA in these chromosomes. We show that anti-Z-DNA antibodies bind to polytene chromosomes in a very reproducible pattern, exhibiting fluorescence staining exclusively in interband regions in our experimental conditions. This finding may throw some light on the role of Z-DNA in biological systems and may lead to some clues concerning the significance of banding in eukaryotic chromosomes.

#### Characterization of the antibody

As described earlier<sup>9</sup>, the anti-Z-DNA antibodies were raised by injecting rabbits with brominated poly(dG-dC)·poly(dG-dC). This DNA polymer remains in the Z-form even in low salt because most of the bromine atoms are on the C-8 position of guanine, stabilizing guanine in the *syn* conformation. In low salt, non-brominated poly(dG-dC)·poly(dG-dC) is in the B conformation and does not react with the antibody. In 4 M NaCl the unmodified poly(dG-dC)·poly(dG-dC) is converted to the Z form and does react with the anti-Z-DNA antibody.

Although our initial experiments on polytene chromosomes were carried out with whole sera from rabbits injected with brominated poly(dG-dC)·poly(dG-dC), the more recent experiments have been done with antibodies purified from these sera. The purified antibody was obtained by preparative scale quantitative immunoprecipitation in 4 M NaCl using unmodified poly(dG-dC)·poly(dG-dC), which is in the Z conformation at that salt concentration<sup>2,4</sup>. The specificity of the affinity-purified antibody for the Z conformation was tested on native *Escherichia coli* DNA and three different polymers, poly(dG-dC)·poly(dG-dC), brominated poly(dG-dC)·poly(dG-dC)·poly(dG-dC) and the methylated polymer poly(dG-dC)·

Fig. 1 Specificity of the affinity-purified anti-Z-DNA antibody as measured by direct binding to <sup>3</sup>H-brominated poly(dG-dC) poly(dG-dC) (①), <sup>3</sup>H-poly(dG-m<sup>5</sup>dC) poly(dG-m<sup>5</sup>dC) (△), <sup>3</sup>H-poly(dG-dC) poly(dG-dC) dC) (O) and <sup>3</sup>H-labelled E. coli DNA (A) in 0.2 M NaCl (a), 1.5 M NaCl (b) and 4.0 M NaCl (c). The affinity-purified antibody was prepared by a modification of the procedure of Kitagawa and Okuhara<sup>22</sup>. A sample of rabbit serum was incubated with an equivalent amount of poly(dGdC) · poly(dG-dC) (as determined from quantitative precipitation curves) in 60 mM sodium phosphate, 30 mM EDTA, 4 M sodium chloride, pH 8.0, for 3 h at 37 °C and overnight at 4 °C. For equivalent proportions of antigen and antibody,  $75 A_{260}$  units of polynucleotide were added to 15 ml of serum. Resulting precipitates were washed three times with cold PBS (10 mM sodium phosphate, 140 mM sodium chloride, pH 7.4) and dissolved in 20 mM sodium carbonate, 5% dimethyl sulphoxide (DMSO) pH 10.5. The dissolved antibodies and antigen were separated on a DEAE-cellulose column (20 ml) previously equilibrated with 20 mM sodium carbonate, 5% DMSO, pH 10.5. The free antibody flowed through on washing with 20 mM sodium carbonate, 5% DMSO, pH 10.5. The retained nucleic acid was eluted by 20 mM sodium carbonate, 5% DMSO, 1 M NaCl, pH 10.5. The purified antibodies were dialysed against PBS and protein concentration determined by the method of Lowry et al.<sup>23</sup>. Radioimmunoassays (RIAs) were performed by incubating  $100\,\mu l$  samples of purified antibody serially diluted into the RIA buffer (60 mM sodium phosphate, 30 mM sodium EDTA, pH 8.0) containing 0.2 M NaCl (a), 1.5 M NaCl (b) or 4.0 M NaCl (c) with 100 ng of <sup>3</sup>H-labelled nucleic acid in 0.05 ml of the RIA buffer containing the indicated NaCl concentration for 1 h at room temperature. Then 0.05 ml of the y-globulin fraction of goat anti-rabbit immunoglobulin serum in PBS was added and the mixture kept at room temperature for an additional hour. The resulting precipitate was centrifuged, washed twice with RIA buffer containing the indicated NaCl concentration, dissolved in 1 ml 0.1 N NaOH and counted in 10 ml of Aquasol-2 (NEN). 3H-thymidinelabelled E. coli DNA was prepared from a mutant strain B3, as described previously<sup>24</sup>, but with a pulse of 0.5 mCi of <sup>3</sup>H-thymidine. <sup>3</sup>H-labelled poly(dG-m<sup>5</sup>dC) poly(dG-m<sup>5</sup>dC) was synthesized according to Behe and Felsenfeld<sup>11</sup>, except that 0.065 mCi of <sup>3</sup>H-dGTP (Amersham) was included into the synthesis reaction mixture. The other polymers were radiolabelled by nick translation<sup>25</sup>.

poly(dG-m<sup>5</sup>dC). Behe and Felsenfeld have shown that methylation on the 5 position of cytosine yields a polymer that is converted to Z-DNA at low concentrations of MgCl<sub>2</sub>, and has a midpoint for conversion by NaCl at 700 mM (ref. 11).

Figure 1 demonstrates the specificity of the purified antibody by showing its direct binding as a function of antibody concentration in three different salt environments using the four polymers. In 0.2 M NaCl, the antibody bound only the brominated poly(dG-dC) · poly(dG-dC) (Fig. 1a), the only one which has the Z conformation in 0.2 M NaCl. At 1.5 M NaCl, antibody bound the brominated and methylated polymers, both of which have the Z conformation, but not the two polymers which are in the B conformation at that ionic strength (Fig. 1b). In 4 M NaCl the brominated. methylated and unmodified dC) poly(dG-dC) were bound, but E. coli DNA was not (Fig. 1c). Poly(dG-dC) poly(dG-dC) is completely in the Z-DNA conformation in 4 M NaCl, where its Raman spectrum is identical with that of Z-DNA crystals4. The three experiments shown in Fig. 1 strikingly illustrate the effect of antigen conformation on the reactivity of the antibody. Only when the polymers are in the Z conformation does the affinity-purified antibody combine with the DNA antigen. When the antigen is not in the Z conformation, no reaction occurs. The same results were obtained with whole serum.

Further analyses on the specificity of the antibody were done by competitive radioimmunoassays in which the ability of other polymers to inhibit the binding of the antibody to a known form of Z-DNA was measured. No inhibition was seen with poly(dG), brominated poly(dG), poly(dG) poly(dC), brominated poly(dG), native and denatured calf thymus DNA, Vero RNA, DNA-RNA hybrids or double-stranded RNAs. All our experiments indicate that the antibody is highly specific for Z-DNA and does not recognize B-DNA or other nucleic acid conformations.

# Binding of antibodies to polytene chromosomes

Identical results were obtained when either the antibodies in whole serum or the affinity-purified antibodies were used to look for the presence of Z-DNA in polytene chromosomes from larval salivary glands of *D. melanogaster*. Antibodies bound to the chromosome are detected by the binding of a second antibody, a fluorescein-labelled goat antibody to the rabbit immunoglobulin. The second antibody also provides an amplification of the binding of the primary antibody, as several molecules of the goat antibody can bind to each rabbit antibody.

The anti-Z-DNA antibody stains the polytene chromosomes in a very distinct and specific way (Fig. 2a). It can be seen that there are many fluorescent regions on all the chromosome arms as well as a somewhat diminished and diffused staining in the chromocentre, where the arms are joined. All the control experiments described above have indicated that the antibody is recognizing the Z conformation. Other control experiments indicate that the antibody binding to the polytene chromosomes is also the antibody that recognizes the Z conformation (Fig. 2b,c). For the preparation shown in Fig. 2b the antibody was incubated with B-DNA, in the form of poly(dG-dC) poly(dGdC), before it was put on the chromosome preparation. The antibody staining of the chromosomes is not affected. However, when the antibody was preincubated with Z-DNA in the form of brominated poly(dG-dC) · poly(dG-dC), the chromosomal staining is completely abolished (Fig. 2c), and chromosomes appear uniformly dark. Note that preparations stained with antibody preincubated with Z-DNA reproducibly show more background fluorescence than do other preparations. This is because the antigen-antibody complex has precipitated throughout the field and around the outside of the chromosomes. This complex has not been washed away and has thus reacted with the fluorescent goat anti-rabbit antibody. Experiments in which the antibody was preincubated with either

native or denatured DNA from *Drosophila* embryos showed no effect on chromosomal staining.

The same chromosomal staining pattern is produced by whole serum from rabbits injected with brominated poly(dGdC) poly(dG-dC), by antibodies affinity purified from this serum by the brominated polymer in low salt, or by antibodies affinity purified from the serum by unmodified poly(dGdC) poly(dG-dC) in high salt where this polymer is in the Z conformation. We have also studied an antibody obtained from a rabbit injected with unmodified poly(dG-dC) poly(dG-dC), which exists as B-DNA in physiological salt solution. The antibody produced by this rabbit was of lower titre but was specific for Z-DNA at the dilution used in the experiment. To obtain this antibody, the polymer was injected into the rabbit complexed with methylated bovine serum albumin and it is assumed that the cationic charges on the surface of the methylated bovine serum albumin provided regions which converted local domains of the poly(dG-dC) · poly(dG-dC) into Z-DNA9. This last antibody produced without bromine atoms yielded the same chromosomal staining pattern as the other antibody

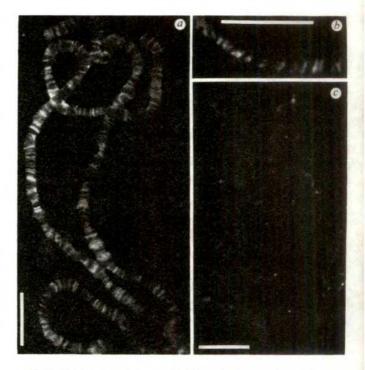


Fig. 2 Fluorescence micrographs of polytene chromosomes demonstrating the specificity of the reaction between anti-Z-DNA antibody and fixed chromosomes. a, Chromosomes stained with anti-Z-DNA antibody by our standard procedure. b, Chromosomes stained with anti-Z-DNA antibody, which had been preincubated with poly(dG-dC)-poly(dG-dC) in the B conformation. The pattern of fluorescence staining is identical to that produced by the antibody without B-DNA competitor. c, Chromosomes stained with anti-Z-DNA antibody, which had been preincubated with the Z-form brominated poly(dG-dC) poly(dG-dC). No chromosomal fluorescence staining is detectable although the precipitated antigen-antibody complex produces an increased general background of fluorescence. Salivary glands were dissected out of third instar Oregon R larvae (gt-1 stock) into a droplet of 45% acetic acid in Ringer's buffer on a coverslip. After 2-5 min a microscope slide was touched to the drop on the coverslip, the gland was squashed mechanically and the slides were placed on the flat surface of a block of dry ice. After 20 min freezing on dry ice the coverslips were pried off and the slides were briefly immersed horizontally in PBS. Excess PBS was removed from the slide without completely drying the surface; 0.02 ml antibody-containing PBS solution was then added and incubated for 15 min at 37 °C. The slides were then gently washed in PBS and fluorescein isothiocyanate (FITC) conjugated IgG fraction of goat anti-rabbit serum (Cappell) was added at a 1:250 dilution in PBS, followed by another 37 °C incubation for 15 min. Unbound FITC-conjugated secondary antibody gently washed out and a clean coverslip was mounted with a drop of PBS. The slides were viewed and photographed in a Zeiss microscope using both incident UV illumination and phase contrast optics. Photographs were taken using Kodak Ektachrome 400 film. For the blocking experiments of b and c, the antibody solutions contained the respective competitor DNAs at concentrations of 0.5 mg ml-1 and were preincubated for 30-45 min at 37 °C before staining of the polytene chromosomes. Scale bar, 10 µm.

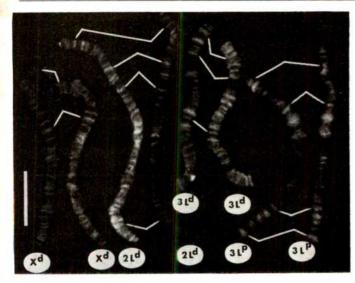


Fig. 3 Fluorescence micrographs of chromosomes stained with the anti-Z-DNA antibody demonstrating the constancy of the fluorescence pattern from nucleus to nucleus. Sections of chromosomes from different nuclei are aligned and selected fluorescent regions are marked to orient the viewer. d marks the most distal sections of chromosome arms X, 2L and 3L; p designates the proximal part. Fluorescence staining and visualization was done as described in Fig. 2. Scale bar, 10 μm.

preparations. None of the normal rabbit sera which we have used nor incubation with fluorescent labelled goat anti-rabbit antibody alone has produced staining of the polytene chromosomes. Thus, all our control experiments indicate that the anti-body is recognizing the Z-conformation in the polytene chromosomes, as it does in the immunoprecipitation experiments.

The anti-Z-DNA antibodies produce a distinctive pattern of staining on the polytene chromosomes; the fluorescent segments seen on the chromosome vary both in size and intensity, but the size and staining intensity of any particular region is reproducible and appears to reflect the nature of the chromatin. Figure 3 shows several examples of chromosomal segments from different nuclei. The reproducibility of the staining pattern is most conveniently seen when the two homologues that make up a chromosome have become unpaired along a part of their length. Figure 4 shows two examples that demonstrate the similarity of the staining pattern in the asynapsed chromosomes.

Some of the fluorescence photographs in Figs 2–4 were exposed for long periods to give a stronger fluorescent signal. However, photographs were also taken with a shorter exposure so that the image would be in the linear range of the film response, and here, the darker non-fluorescent segments were as dark as the general background. From analyses of negatives of these photographs using a densitometer tracing to look at the variations in the amplitude, we estimate that the intensity of different fluorescent segments roughly varies by a factor of 5–10 in brightness.

To determine which segments of the *Drosophila* chromosome are fluorescing, we compared the pattern of bands seen in phase microscopic photographs with the fluorescence pattern (Fig. 5). Photographs of segments from various parts of the *Drosophila* chromosome were cut along the chromosomal axis and the phase photographs were compared with the fluorescence photographs. Figure 5 shows that the fluorescent segments are the interbands and not the bands. The bright areas in the fluorescence photograph are found in the position of the light interband regions in the phase photographs. Note that there is not necessarily a correlation between the width of the interband segment and its fluorescent intensity.

Thus, the antibody binding results shown in Fig. 1, together with the negative controls, clearly demonstrate that we are using an antibody that is specific for the Z-DNA helix. It reacted with three polymers only in distinct conditions required for each to occur in the Z conformation, and did not react with E. coli DNA in any of these conditions, nor with single-stranded DNA and a

variety of polynucleotides, including brominated polymers. The bromination was not required either for induction of Z-specific antibody or for its serological reactivity. The antibody that binds to polytene chromosomes shows the same properties as those measured in the binding experiments; chromosomal staining is blocked only by molecules that have a Z conformation and yield an immune precipitate with the antibody.

## Tests for effects of experimental procedures

As the chromosomes in almost all cytological preparations have been subjected to treatments that remove or alter cellular components, this raises the question of whether sample preparation induces formation of Z-DNA in the polytene chromosomes. At present, the only way we can approach this question is by testing the effects of various preparative procedures, and we have therefore tested preparations from salivary glands that were incubated for 2-5 min at room temperature in 45% acetic acid, squashed in the same solution, frozen on dry ice and treated in various ways before incubation with antibody. After freezing and removal of the cover slips, the slides were: (1) placed directly in phosphate-buffered saline (PBS), or (2) immersed in ethanol, air dried and then placed in PBS, or (3) placed directly in a post-fixative solution containing 3.7% formaldehyde and then in PBS. Alternatively, glands were fixed by the formaldehyde technique of Silver and Elgin<sup>12</sup> squashed in 45% acetic acid with 10 mM MgCl2 and frozen on dry ice, then immersed in ethanol and air dried before incubation with antibody in PBS.

These treatments might be expected to produce different types of artefact, yet all result in qualitatively the same staining pattern. The first procedure produces by far the most intense staining pattern and has been used as our standard preparative technique. Less intense staining occurred when formaldehyde was used for fixation and so further study will be required to determine whether bright staining was due to selective removal of proteins during exposure to 45% acetic acid. In the post-fixed preparations it is likely that the formaldehyde is itself blocking the antibody binding site.

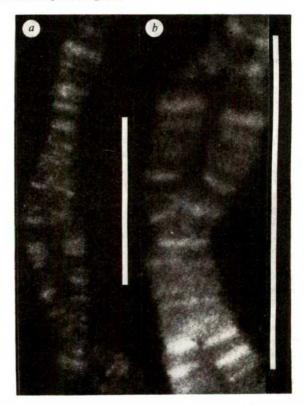


Fig. 4 Reproducibility of the anti-Z-DNA antibody staining pattern as seen in two examples of asynapsis (a, b). The unpaired homologues of a polytene chromosomal arm show very similar fluorescence staining in the homologous regions. Experimental protocol was as described in Fig. 2.

The polytene chromosome is segmented into bands and interbands. The bands vary in size, yielding a banding pattern that is invariant unless chromosomal rearrangements occur. These banding patterns are useful for genetic mapping studies and for studies of evolutionary relationships between species; however, their role in chromosome structure is unknown. Banded polytene chromosomes have also been reported in several other eukaryotic organisms including protozoa and plants13. It has been suggested that this banding might be a general property of the organization of the eukaryotic chromosome<sup>13</sup>. Drosophila has ~5,000 bands as well as interbands which have been identified and mapped. On average, a band plus interband contains some 30 kilobases of DNA<sup>13</sup>. The DNA in the interband has been estimated as ranging from 5% (ref. 13) to 30% (ref. 14) of the total DNA, depending on the method of measurement. Electron micrographs of Drosophila chromosomes show bands as containing darkly staining, densely clumped units while the interbands are visualized as elongated fibrils 50-60 Å in diameter13. Indirect immunofluorescence has revealed a number of proteins in the bands 15-17 and interbands 16-18

An important question in any immunofluorescence experiment is whether the antigen is present but not accessible for reaction. This question is especially relevant to our experiments because staining is limited to the least compact regions of the chromosomes. Although anti-histone antibodies can cause brighter staining in the more compact regions than in interbands is it is possible that the DNA, or a particular portion of it, is not as available as the histones. Although we cannot rule out the presence of Z-DNA in the band regions, our experiments do show that it is either more abundant or more accessible in the interbands. Either of the alternatives is interesting and a decision between the alternatives may help explain the significance of chromosomal banding. This question is under further study with antibodies that react with other forms of DNA.

The reproducibility and uniformity of the staining pattern throughout the entire length of the polytene chromosome are remarkable. We have compared the staining pattern among different giant polytene chromosomes within an individual and between individuals. The staining pattern of Z-DNA antibodies appears to be a constant feature of the chromosomes in much the same way that the banding in the phase contrast microscope appears constant. This suggests that the pattern is an intrinsic property of the chromosome rather than, for example, a property induced through the mechanical action of squashing the chromosome, for then one would expect significant differences in the staining pattern in various parts of the chromosome, depending on the degree of mechanical stress to which each segment was subjected.

The intensity of staining in different interbands varies considerably, and seems to be unrelated to the width of the interband; the origin of the variability is not known. The possibility that the brighter segments contain a larger number of closely packed and unresolved band-interband units is unlikely, because we have occasionally seen extremely stretched regions in which the interbands are considerably extended and still do not break up into smaller fluorescent segments.

It would be interesting to know the proportion of the interband DNA that needs to be Z-DNA to produce the observed fluorescent pattern. This is difficult to estimate because we have no absolute standard which will allow us to determine accurately the amount of primary antibody bound from the amount of fluorescence detected. As a lower limit it is possible that only a small stretch of some 6–8 base pairs out of the 3 kilobase pairs in the average interband might be in the Z conformation. In a polytene chromosome, where 1–2,000 copies of such a sequence are lying in register, one would have enormous numbers of antibodies arrayed transversely across the interband. Because of the limited optical resolution in the light microscope and the broadening of the fluorescent image, one site would appear to occupy the entire 0.1 μm of an average interband. As an upper

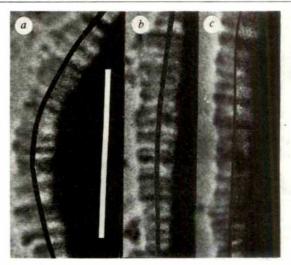


Fig. 5 Comparison of phase contrast and anti-Z-DNA antibody-stained fluorescent photographs of polytene chromosomes. Chromosome sections from three different nuclei (a-c) are shown. Analogous sections of the same chromosome are compared by cutting the photographs down the chromosomal axis and comparing the two halves. The light areas of the left phase contrast photographs correspond to the interbands while the darker sections are the bands. The white sections of the right darkfield fluorescent photographs show the position of the anti-Z-DNA fluorescent stained antibodies. It can be seen that the interbands are fluorescing. Note that the intensity of fluorescence is not necessarily related to the width of the interband. Fluorescence staining was done as described in Fig. 2 legend. Scale bar, 5 μm.

limit, the entire interband could be in the Z-DNA conformation. Although this seems implausible, further work will be required before we can make a more accurate estimate.

## Possible functional significance of Z-DNA

Because the Z conformation is a reversible structural form of DNA it is an attractive candidate for having a regulatory role in genetic activity. Many regions of the polytene chromosomes undergo changes in chromatin structure, called puffs, which are associated with transcription of certain genes. The patterns of Z-DNA staining during such structural changes will be of obvious interest.

In physiological salt conditions the Z-DNA conformation is somewhat less stable than the B conformation and therefore has to be stabilized. There may be four ways of maintaining the Z conformation in biological systems. (1) Supercoiling. Z-DNA twists the double helix in a left-handed mode, opposite to the right-handed B-DNA conformation. Because of this one can interchange strongly negative supercoiled DNA for segments of Z-DNA (L. Peck, A. N., A. R. and J. C. Wang, in preparation). (2) Binding to proteins which are specific for the Z conformation. These probably involve electrostatic interactions with basic residues. (3) Binding to specific ions. For example, spermine or spermidine can stabilize crystals of Z-DNA so that they form with a regularity which yields an atomic resolution diffraction pattern<sup>1,7</sup>. (4) Modification such as methylation of . (4) Modification such as methylation of cytosine in the 5 position (although not necessarily applicable to Drosophila DNA19). In the absence of methylation poly(dGdC) poly(dG-dC) requires 700 mM Mg2+ to convert to the Z conformation. Behe and Felsenfeld11 have shown that when the methyl group is present, the Z conformation is formed in 0.6 mM Mg<sup>2+</sup>. It is interesting that spermine is even more effective, forming the Z conformation at a concentration of 0.002 mM. Further work must be done before we fully understand the parameters which stabilize the Z conformation in the polytene chromosome.

Work with synthetic deoxypolynucleotides has amply demonstrated that there is a reversible equilibrium between the right-handed B conformation and the left-handed Z conformation in molecules in which there is an appropriate sequence. Recent work with cloned segments of poly(dG-dC) poly(dG-dC) in plasmids have demonstrated that it is possible for these segments to undergo a B to Z transformation even though they

are enclosed in plasmid segments containing B-DNA (ref. 20 and L. Peck, A. N., A. R. and J. C. Wang, in preparation). It is thus reasonable to imagine that appropriate segments in chromatin might undergo similar changes in conformation. Z-DNA segments may have the property of not only changing the local environment near a particular gene, but through interaction with supercoiling these segments could modify the transcribability of DNA regions far removed from the site at which the Z-DNA segment is found.

The striking observation here is that an antibody specific to left-handed Z-DNA binds in a regular fashion to the interband regions of the D. melanogaster polytene chromosomes. Our control experiments strengthen the conclusion that this binding

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is due to a Z-DNA conformation found in the interbands. Band-interband units seem to be basic units of gene activity<sup>13</sup> replication<sup>21</sup> and perhaps chromosomal synapsis. It is tempting to suppose that Z-DNA might be a conformational switch which is involved in the control of transcription or of other of these activities. As Z-DNA appears to be a fundamental component of the polytene chromosome, these chromosomes may provide a unique opportunity for further exploration of the role of Z-DNA.

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## **Dual function transcripts specifying** tRNA and mRNA

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A cluster of four tRNA genes in Escherichia coli is co-transcribed with an adjacent gene encoding elongation factor Tu. The resultant transcript that specifies both structural (tRNA) and informational (mRNA) RNA may not be an uncommon occurrence and has interesting regulatory implications.

IN Escherichia coli, where regulation of transcription has been most extensively studied, the RNAs synthesized are generally divided into two categories—the class of informational or mRNAs that are metabolically unstable, and the class of structural RNAs such as rRNA and tRNA that do not show appreciable turnover. The two classes are also distinguishable by their regulation pathways. In particular, stable RNA is subject to a coordinate regulation that is responsive to the availability of amino acids for protein synthesis. In general, this type of control only affects informational RNAs encoding proteins required in protein synthesis.

Transfer RNA genes are distributed throughout the E. coli genome as part of ribosomal RNA operons or as clusters of tRNA genes containing either different tRNA genes or repeats of the same gene (for review see ref. 1). We report here a novel transcription unit composed of a tRNA cluster and mRNA and suggest that the co-transcription of structural and informational RNA may not be an uncommon occurrence. Possible implications of these dual function transcripts are also discussed.

## Organization and structure of the tyrU gene cluster

The tyrU gene cluster is situated between the rmB ribosomal operon and the tufB gene at 89 min (ref. 2). The tyrU locus encodes the major tyrosine accepting species, tRNA2Tyr, which

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occurs as a single copy within a cluster of three other tRNA genes<sup>3-5</sup>. The tufB locus codes for elongation factor (EF)-Tu, which promotes the binding of aminoacyl-tRNA to ribosomes. In addition to its key role in translation, EF-Tu functions as a structural component of  $Q\beta$  replicase (see ref. 6 for review). Figure 1 shows the DNA sequence of the tyrU gene cluster

and the 5' end of the adjacent tufB gene, which is located only 114 base pairs (bp) downstream of the tRNA cluster. The tyrUpromoter (as identified by hybridization analyses presented below) is located directly upstream of the first tRNA gene in the cluster and shares two features with the tyrT promoter region: a region of G-C bias flanking the Pribnow box (Fig. 1 and ref. 7), and wo dyad symmetries, one involving the -35 region and the other centred near the Pribnow sequence (Fig. 1 and ref. 8). These sequence features are present in the promoter regions of other stringently controlled genes and may therefore have a role in their coordinate synthesis. The Pribnow box participates in a dyad symmetry in the ribosomal protein operons and the G+C-rich sequence following the Pribnow heptamer has been noted for rRNA and ribosomal protein operons<sup>10</sup>.

## A co-transcript of tRNA and mRNA

Knowledge of the organization and structure of the tyrU cluster and adjacent tufB gene has facilitated analyses of the in vivo transcripts and processing intermediates. The methods developed by Alwine and co-workers<sup>11</sup> were used to detect RNA species that comprise a small fraction of the total cellular RNA. The experimental approach involved fractionating denatured RNA by gel electrophoresis, transferring the RNA to diazobenzyloxymethyl (DBM) paper and probing the RNA blots with radiolabelled restriction fragments isolated from the tyrU region (as detailed in Fig. 2 legend). Detection of RNA precursors was maximized by using E, coli strains deficient in RNA processing. Precursor tRNAs accumulate in these strains as a result of mutations blocking and/or delaying their processing<sup>12</sup>.

Figure 2 shows results of this analysis for selected probes. The tRNA-specific probe (Fig. 2 B) hybridized to one band of 1,800 bases (transcripts smaller than  $\sim$ 700 bases were detected on the higher composition gels shown in Fig. 5). The tufB probe (Fig. 2 C) also hybridized to the 1,800-base transcript, as well as to four other transcripts sized at 4,700, 1,550, 1,420 and 1,290 bases. As detailed below, the 4,700-base transcript contained sequences from the other gene encoding EF-Tu, tufA (ref. 13), and the lower molecular weight transcripts probably represent tufA and tufB processing intermediates. The probes immediately upstream (Fig. 2 A) or downstream (Fig. 2 D) of the tRNA-tufB region did not hybridize to the 1,800-base transcript. This collection of probes thus defines a transcript of 1,800 bases which contains sequences from both the tRNA gene cluster and the tufB gene.

The 1,800-base transcript was further characterized by

sequential hybridizations using probes covering different portions of the tyrU region. Figure 3 summarizes the hybridization results. Eight probes isolated from regions of structural tRNA or tu/B sequence displayed hybridization to the 1,800-base transcript. Importantly, the sum of the restriction fragments that hybridized to the co-transcript corresponds to the molecular weight of the transcript (1,800 bases) determined on denaturing gels. Probes that failed to hybridize to the 1,800-base transcript defined the outer limits of the transcript: the 5'-proximal probe that produced negative results was separated from the structural tRNA<sub>4</sub><sup>Thr</sup> sequence by 12 bp and the 3'-proximal probe began 85 bp downstream of tu/B structural sequence. Thus, this series of probes specified a transcript encompassing both the tRNA cluster and the tu/B gene.

To establish these unexpected results unequivocally, control experiments were carried out to ensure that: (1) failure to detect hybridization with the negative probes was not due to insufficient radioactivity, and (2) the observed hybridization with the positive probes was not the result of either contamination (with other labelled DNA) or the result of fortuitous homologies. The ability of each nick-translated probe to produce a strong hybridization signal to its respective band and to no other fragments of a digest of  $\lambda$  riff 18 DNA was tested. As

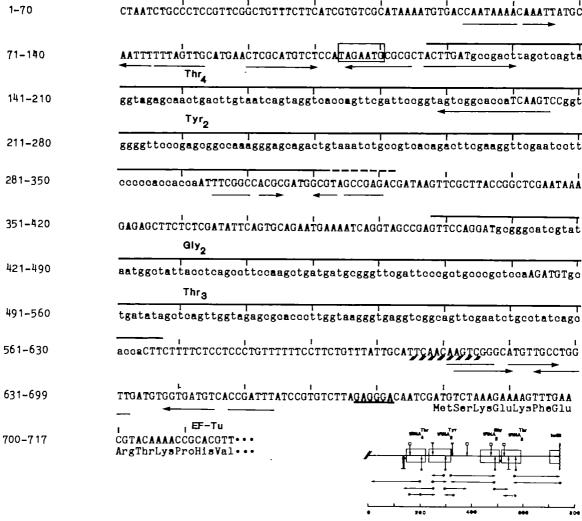


Fig. 1 Nucleotide sequences of the tyrU cluster and proximal ta/B region. The DNA strand with the sequence of the RNA transcript is shown. The source of DNA was the transducing phage λ rm<sup>4</sup>18 (ref. 51; supplied by M. Nomura or N. Fill). DNA sequencing was by the method of Maxam and Gilbert<sup>52</sup>; \*, 5'-labelled end of the restriction fragments; →, direction and extent of the sequence obtained, the scale is in base pairs. Complement and overlap were obtained for all new sequences reported here with the exception of the sequence upstream of the tRNA<sub>4</sub><sup>The</sup> gene. The sequence is identical to that reported by An andProsen<sup>16</sup> with the exception of an additional base pair they place at position 317. Structural tRNA sequence (lower case) and the two dimeric procursors are overlined. The tRNA<sub>2</sub><sup>Che</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub><sup>The</sup>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-(RNA<sub>4</sub>-

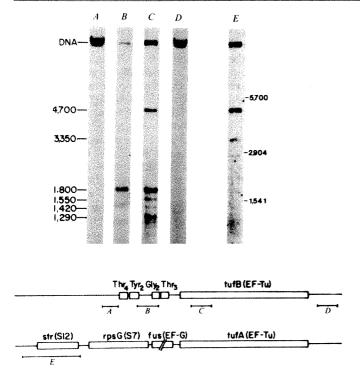


Fig. 2 Characterization of the transcript specifying the tyrU cluster and tufB. E. coli strain ABL-1 (RNase III RNase Pt; ref. 55; gift of W. McClain) was grown for several generations at 30 °C, and then incubated at 42 °C for 30 min to inactivate RNase P. RNA was extracted according to Gegenheimer and Apirion<sup>55</sup> as detailed elsewhere<sup>30</sup>, denatured with glyoxal<sup>56</sup>, electrophoresed in 1.5% agarose gels with recirculation of the 10 mM sodium phosphate, pH 6.5, 1 mM EDTA running buffer, and transferred to DBM paper<sup>11</sup>. The RNA blots were probed with DNA fragments (A-E) which were nick translated<sup>57</sup> with [ $\alpha$ - $^{32}$ P]dCTP (NEN; 2-3,000 Ci mmol<sup>-1</sup>) to a specific activity of  $\sim 5 \times 10^7$  c.p.m. per µg. Hybridizations were carried out in the presence of 10% dextran sulphate as detailed by Alwine *et al.*<sup>11</sup> but omitting carrier DNA. Blots were washed first in 0.3 M NaCl, 0.03 M sodium citrate, 0.1% SDS at 23 °C for 20 min, and then in 15 mM NaCl, 1.5 mM sodium citrate, 0.1% SDS for 45 min at 50-55 °C. The 32P-labelled probes isolated from the region of  $\lambda$  rif<sup>d</sup> 18 depicted in the diagram were: A Hhal-145; B, Hintl-190; C, Hincll-Smal-187; D, BstNl-HaeIII-185. Probe E is a HaeIII-530 fragment encompassing the S12 ribosomal protein gene, which was isolated from \( \lambda \) fus3 (obtained from J. Yates and M. Nomura). Molecular weight markers were 16S (1,541 bases), 23S (2,904 bases) and 30S RNA ( $\sim$ 5,700 bases). Lanes B, C and E have  $\sim$ 2 µg RNA per lane, and lanes A and D have 30  $\mu$ g per lane. The scale is in bases. Structural sequences for tRNAs and tufB are boxed.

shown in Fig. 4 for selected probes and detailed in the legend, each probe hybridized only to its corresponding  $\lambda$  rif<sup>d</sup>18 restriction fragment.

The hybridization results place the promoter for the tRNAtufB co-transcript directly before the tRNA4 gene and the terminator closely following the tufB structural sequence. Note that these assignments are not precise, because a probe could share as much as 30 bases of homology with the transcript and fail to hybridize. Genetic evidence corroborates the location of the tufB promoter directly upstream of the tRNA cluster<sup>60</sup>. In addition, Taylor and Burgess<sup>14</sup> found that a restriction fragment containing the presumptive tRNA-tufB promoter (labelled HaeIII-890 by Taylor and Burgess<sup>14</sup> and HaeIII-700 by Rossi et al. 15) formed filter-retainable complexes with RNA polymerase. Finally, inspection of the DNA sequence reveals a promoter region situated directly before the tRNA<sub>4</sub><sup>Thr</sup> gene (see Fig. 1), a location consistent with the analyses of the functional promoter. The location of a possible termination sequence 34 bases following tufB (ref. 16) is also consistent with our hybridization results (Fig. 3). That termination occurs at this site in vivo is also suggested by the presence of gene U, which is transcribed from its own promoter ~200 bases downstream of tufB (refs 16, 17).

## Other EF-Tu transcripts

EF-Tu is encoded by duplicate genes that contribute approximately equivalent amounts of EF-Tu to the cell<sup>18,19</sup>. The two proteins differ only in the carboxy-terminal amino acid<sup>19</sup>, suggesting that they are functionally equivalent. The other gene for EF-Tu, tufA, is located at 72 min as the terminal gene of the str ribosomal protein operon<sup>13</sup>. The str-rpsG-fus-tufA genes are transcribed from a single promoter<sup>13</sup> located 80 bp upstream of str structural sequence<sup>20</sup>, which could yield a primary transcript with an estimated size of 4,800 bases.

Probes prepared from tufB structural sequences hybridize to both tufA and tufB transcripts, as the DNA sequences of tufA and tufB differ at only 13 of 1,181 bp<sup>16,21</sup>. The largest tuf transcript was suspected to be tufA specific, as probes immediately upstream and downstream of the tRNA-tufB region failed to hybridize to it (Fig. 2). A probe isolated from the 5' end of the str operon was used to prove that the 4,700-base transcript encoded the str operon. The str probe hybridized to the 4,700base transcript that was also detected with the tufB structural probe (Fig. 2 E), indicating that the 4,700-base transcript corresponds to a full-sized str operon mRNA. This same probe also hybridized to a 3,350-base message that did not hybridize with tufB structural probes and could therefore encode the first three genes of the str operon (str-rpsG-fus). Our present data do not indicate whether the three smallest RNAs (1,290, 1,420, 1,550 bp (originate from tubB or tufA or both. As determined by densitometric tracings, these small RNAs are less abundant than the major 1,800-base transcript (by 2-, 22-, and 6-fold for 1,290-, 1,420- and 1,550-base transcripts respectively).

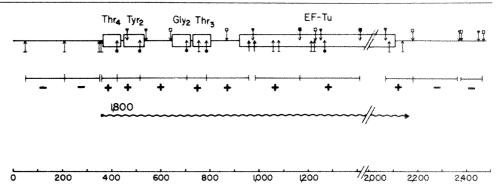
## Modes of EF-Tu synthesis

The presence of one copy of EF-Tu in a ribosomal protein operon and the other in a tRNA operon may be important in coordinating the synthesis of these three components of the translational machinery. The intracellular concentration of EF-Tu does, in fact, parallel that of tRNAs and ribosomes during changing growth conditions in *E. coli*<sup>22-24</sup>. Moreover, EF-Tu synthesis from both loci is subject to stringent control in response to amino acid starvation<sup>23,25-28</sup>.

In addition to transcripts corresponding to full-size str operon (4,700 bases) and tRNA-tufB operon (1,800 bases), there are three smaller tuf messages (1,290, 1,420 and 1,550 bases) (see Fig. 2). The 1,290-base message corresponds to the non-tRNA cleavage product of the 1,800-base primary transcript as shown in Fig. 5. In addition, an RNA of this size could result from transcription initiating at a promoter site (TGATGTC) located 32 bases upstream of tufB. Indeed, some promoter activity has been observed in this region in vivo 60. The intergenic region also contains a possible termination sequence (CAACAA; refs 29, 30) that overlaps a sequence (TTCAACAAGTC) postulated to be involved in processing because of its homology with the characterized tyrT processing site (ref. 31; see also Fig. 6). Due to this overlap the alternative, non-mutually exclusive pathways that could uncouple EF-Tu synthesis from that of the tRNA cluster (processing versus termination and re-initiation of transcription) cannot be distinguished. However, the presence of large amounts of the 1,800-base transcript (Fig. 2) indicates that termination following the tRNA cluster (if it occurs at all) cannot be very effective in these growth conditions.

A similar potential for uncoupling exists at the *tufA* locus. The 3,350-base *str-rpsG-fus* message (Fig. 2) could result from either a termination or processing event or both. Analogous to the *tufB* region, promoter activity of DNA segments directly upstream of *tufA* has been observed *in vivo* (ref. 21 and J. D. Friesen and co-workers, personal communication). As noted below, the processing site conserved between the *tyrT* and *tyrU* transcripts is also present in the *fus-tufA* intergenic region, a finding that favours processing as a mode of uncoupling. Consistent with the suggested uncoupling of *tufA* synthesis, Nomura and co-workers find that translational repression of *str* 

Fig. 3 Organization and transcription of the tRNA-tufB region. The locations of the restriction sites were obtained from the sequence shown in Fig. 1 or ref. 16. Hybridization to the 1,800-base transcript (~) is denoted by a + in the appropriate restriction fragment (from Arif<sup>d</sup>18) and lack of hybridization by -The HinfI-82 probe from the tRNA region hybridized strongly to 16S RNA in addition to the 1,800-base transcript. This result is consistent with the presof a number of 12-16-bp homologies between 16S RNA<sup>58</sup> and tRNA<sup>Thr</sup> sequences. Due to the hybridization with 16S RNA, the Hinfl-82 probe could not be used to determine

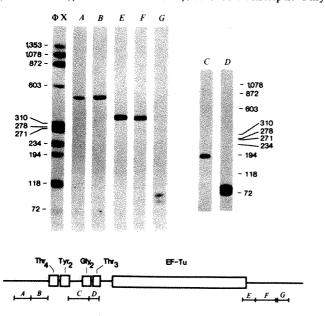


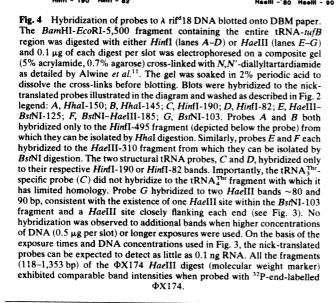
whether the smaller *tuf* transcripts (1,550 and 1,420 bases shown in Fig. 2) were *tufA* or *tufB* specific. The scale is in bases. Structural sequences for the tRNAs and *tufB* are boxed. \$\forall\$, Bst1; \$\div\$, HaeIII; \$\div\$, HincII; \$\div\$, HincII; \$\div\$, Smal.

operon by S7 protein does not inhibit EF-Tu synthesis in vitro 32,33 or in vivo (M. Nomura, personal communication).

## Processing intermediates of the tyrU cluster

Figure 5 gives the tRNA processing intermediates of the 1,800-base primary transcript. Each probe hybridized to mature tRNA (75-85 bases), the 495- and the 1,800-base transcripts. Only





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tRNA<sub>4</sub><sup>Thr</sup> or tRNA<sub>2</sub><sup>Tyr</sup> probes hybridized to a 200-base transcript; similarly, only tRNA<sub>2</sub><sup>Gly</sup> or tRNA<sub>3</sub><sup>Thr</sup> probes hybridized to a 170-base transcript. The data suggest a processing scheme (depicted in the lower portion of Fig. 5) in which the primary transcript is initially processed to a 495-base transcript containing the tRNA cluster and a *tufB* mRNA of ~1,300 bases (Fig. 2). The tRNA precursor is subsequently processed to two dimeric precursors, a 200-base transcript containing tRNA<sub>4</sub><sup>Thr</sup> tRNA<sub>2</sub><sup>Tyr</sup> and a 170-base transcript containing tRNA<sub>2</sub><sup>Gly</sup> tRNA<sub>3</sub><sup>Thr</sup>. Both these dimers are very close in size to those previously isolated from an *E. coli* strain with thermolabile RNase P activity<sup>34-36</sup>.

A secondary pathway for processing the tRNA-tufB transcripts may exist in RNase P-deficient strains. A 415-base transcript was apparent in lanes probed with A, B and C but not D, suggesting that this transcript consists of a tRNA\_T^{Dr}-tRNA\_2^{Gly} trimer. This trimer may result from cleavage of either the 1,800- or 495-base transcript between the tRNA\_2^{Gly} and tRNA\_T^{Dr} structural genes.

The positions of the  $tRNA_2^{Thr}$ - $tRNA_2^{Tyr}$  and  $tRNA_2^{Gly}$ - $tRNA_3^{Thr}$  dimers, based on the size of the processing intermediates (Fig. 5) or obtained from the sequence of the  $tRNA_2^{Gly}$ - $tRNA_2^{Thr}$  dimer<sup>34</sup>, are shown in Fig. 1. The long spacer separating the two dimers is similar to that found in tyrT (ref. 37) and supB-supE (ref. 38) and may be a common feature of tRNA clusters. The 114-base spacer separating the tRNA cluster and tufB is characterized by a 34-base C+T-rich region directly following the tRNAs.

The endonucleases responsible for cleaving the 1,800-base primary transcript have not been identified. One candidate was RNase III; however, there was no significant difference in the transcripts isolated from strains with or without functional RNase III (ABL-1 compared with A49). Other candidates include the tRNA processing endonuclease(s) partially purified by several laboratories<sup>36,39-41</sup>. It is of interest that the sequence conserved between the *tyrT* and *tyrU* primary transcripts, which is apparently the site for cleaving the tRNA cluster<sup>31</sup> from the distal message (see Fig. 5), is also present in the *fus-tufA* intergenic region<sup>21</sup> (TACCAAAGTC matches in 8 of 10 positions). The existence of a conserved processing site suggests that a single endonuclease may coordinately control EF-Tu processing from the *tufA* and *tufB* transcripts.

Finally, we note that in cells wild type for processing enzymes (CA274), the 1,800-base transcript is the only tyrU precursor detectable; none of the tRNA processing intermediates can be seen. Furthermore, as the amount of 1,800-base transcript in CA274 is comparable with that observed in cells deficient for processing (RNase Pts or the double mutant RNase III RNase Pts), we suggest the tRNA sequences on the 1,800-base transcript are matured and function as tRNA in the cell.

## Generality of dual function transcripts

The presence of tRNA on the same transcript as mRNA may not be rare in prokaryotes. An analysis of the  $in\ viv\theta$  transcripts of the  $tRNA_1^{Tyr}$  gene in  $E.\ coli$  has revealed a 650-base transcript

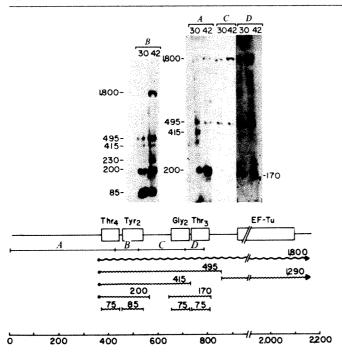


Fig. 5 Processing intermediates of the tyrU cluster. RNA was extracted from E. coli strain A49 (RNase Pts; ref. 59; gift of W. McClain) grown at either 30 or 42 °C, electrophoresed on 2% agarose gels at 30 µg RNA per slot and blotted onto DBM paper. Transfer conditions were similar to those described for Fig. 2 except that glyoxal was removed with a lower concentration of sodium hydroxide (10 mM compared with 50 mM for Fig. 2). Molecular weight markers were 5'-end-labelled *Hae*III and *Hin*cII fragments of  $\Phi$ X174. The gel shown in *B* was electrophoresed for a shorter time to retain mature tRNA. The <sup>32</sup>P-labelled probes isolated from the regions of A rif<sup>d</sup>18 illustrated in the diagram were: A, Hinf1-420; B, Hinf1-93; C, HinfI-190; D, HinfI-82. The putative primary transcript (\*\*), processing intermediates and mature tRNAs (m) are labelled by their respective sizes (in bases) on denaturing gels. The probe containing the structural tyrosine tRNA sequence (B) hybridized to a 230-base transcript that also hybridized with a tRNA<sub>1</sub><sup>Tyr</sup> upstream probe and must therefore be a tyrT transcript. Probe C contained only 18 bases of structural tyrosine tRNA sequence, which severely limited its binding to the 200- or 230-base tyrosine transcripts in the stringent washing conditions used in these experiments. In the blot shown, the hybridization of probe C to the 170-base transcript did not photograph; in other blots this probe detectably bound the 170-base transcript. The processing scheme is discussed in the text

which, in addition to containing two tRNA<sub>1</sub><sup>Tyr</sup> sequences, can code for a small arginine-rich protein<sup>30</sup>. The putative basic protein is encoded by the first of the three repeated units that follow the tRNA<sub>1</sub><sup>Tyr</sup> structural gene<sup>37</sup>. That the *tyrT* transcript actually encodes protein is suggested by the *in vitro* production of a low molecular weight, basic protein that maps to the first repeat<sup>61</sup>.

Transcripts of the tyrT and tyrU gene clusters share several interesting features (Fig. 6): (1) the 5' portion of both transcripts

consists of two regions of cloverleaf secondary structure that are separated by a long spacer (115-208 bases), (2) the tyrosine structural sequences present on each transcript are almost identical, differing by only two bases in the variable loop region <sup>42</sup>, and (3) following the tRNA region there is an intergenic spacer of 67-114 bases containing a conserved sequence (TTCAAAAGTC; located in a region of dyad symmetry) which seems to be the site for processing the tRNA cluster from the mRNA portion of the transcript (ref. 31 and Fig. 5). Finally, the proteins encoded by the two transcripts may share the property of being produced in large quantities by the cell. EF-Tu represents as much as 5% of the total cell protein in E. coli<sup>22,24,43,44</sup>. The basic protein encoded in the tyrT transcript, if actually a DNA-binding protein as suggested by its resemblance to the basic protamines<sup>61</sup>, might also be expected to be relatively abundant.

Co-transcription of tRNA and mRNA has also been observed in other systems. In mitochondria, where transcription initiates at a unique site, almost every protein coding sequence is directly flanked by a tRNA gene<sup>45-47</sup>. Although the co-transcript seems to be very unstable, this arrangement may provide a requisite economy of space in the genome and/or conveniently placed processing signals in the nascent RNA<sup>45,47</sup>. Another system is bacteriophage T4, where a cluster of eight tRNAs is transcribed from the *ipI* promoter located 1,000 bases upstream<sup>40,48</sup>.

## Physiological implications

The tRNA secondary and/or tertiary structure could stabilize adjacent mRNA by retarding the usually rapid 5' to 3' degradation of message<sup>49</sup>. Detection of substantial quantities of intact dual function transcript in cells wild type for processing enzymes is consistent with a stabilization role for the tRNA cluster. If mRNA stabilization is important, the results of Pedersen et al. imply that the tRNAs must be removed before translation. They observed that decay of protein synthetic capacity for tufA and tufB differed by less than 20 (ref. 50). In these experiments, RNA synthesis was inhibited by rifampicin or streptolygidin and it is possible that cleavage between the tRNA and tufB mRNA was also impaired. This would mask any storage form of the transcript that could be activated for translation by cleavage of the proximal tRNA. Alternatively (not mutually exclusive), co-transcription of tRNA and mRNA may be viewed as a mechanism for coordinately controlling the synthesis of key proteins with those of stable RNAs, thereby regulating the production of a particular protein with the growth requirements of the cell.

#### **Conclusions**

Analyses of the structure and transcription of the tyrU locus have revealed a new class of tRNA gene. Transfer RNA genes can be categorized by their proximity and co-transcription with

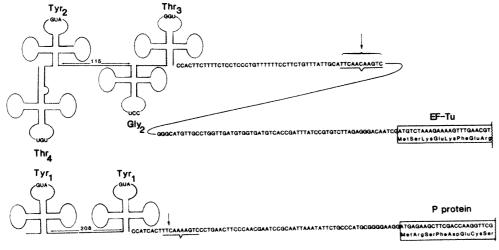


Fig. 6 Comparison of the tyrT and tyrUprimary transcripts. The tyrT transcript was characterized by Rossi et al. RNA extracted from an RNase Pis E. coli strain infected with  $\Phi 80 \text{ psu}_3^{+,-}$  (doublet). The site conserved between the tyrT and tyrU gene clusters and known to be a 3'-processing site for tyrT transcripts31 is underlined. For tufB (ref. 19) and for the protamine-like protein (P protein; see text), the first eight amino acids are indicated. Translation of P protein also initiates at the methionine located 4 codons upstream of the start site shown, as indicated by the in vitro production of both a 29- and 33-amino acid basic protein that map to the first repeat<sup>6</sup>

either rRNA genes, other tRNA genes and/or genes encoding protein. It is tempting to speculate that the tRNA secondary and/or tertiary structure provides an additional level of posttranscriptional control for dual function transcripts. The precise role of the tRNA molecules on the co-transcript remains to be defined.

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## **Recurrent Seyfert** activity in spiral galaxy nuclei

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Hills1 has pointed out that in some conditions black holes in galactic nuclei would rapidly grow by disruption and accretion of stars thus producing Seyfert luminosities (>10<sup>43</sup> erg s<sup>-1</sup>). In this hypothesis only those galactic nuclei with a high central stellar density are susceptible to Seyfert pathology, and they evolve through only one Seyfert phase. Here, I suggest an alternative model for recurring activity in normal galactic nuclei based on the assumed presence of a massive nuclear black hole (  $10^7 M_{\odot}$ ) and the observation of a very clumpy distribution of interstellar gas in the inner 200 pc of our Galaxy. The system of massive molecular clouds with low net angular momentum could provide an 'accretion event' every 10 yr with a duration of ·10<sup>5</sup> yr. In such a picture, most spiral-galaxy nuclei evolve through recurring Seyfert episodes of rather short duration; the 1% accretion duty cycle is roughly consistent with the fact that a few per cent of all spiral galaxies are Seyferts2.

Molecular line observations of the central region of our Galaxy have revealed some  $10^7$ – $10^8\,M_\odot$  of interstellar gas in the inner 200 pc concentrated in massive molecular clouds (ref. 3 and refs therein). The 2.6-mm emission line of carbon monoxide has been particularly useful in mapping the distribution and kinematics of this molecular gas4.5. These studies show that there are essentially two kinematic groups of molecular clouds in the central region (Fig. 1). First, there are about 10 very massive clouds ( $\geq 10^6 M_{\odot}$ ) including the Sgr A and Sgr B2 complexes

which lie entirely at positive longitudes and within a projected distance of 150 pc from the centre. These clouds have line-ofsight velocities which are generally permitted in the sense of galactic rotation but which are much lower than the circular velocity at the sub-central point<sup>6</sup>. Either these clouds are presently stretched along the line-of-sight towards the centre (an unlikely and short-lived configuration), or they are close to the galactic centre but do not take part in systematic galactic rotation—they have a relatively low net angular momentum with respect to the centre.

The second group of clouds seems to lie in a single kinematically connected feature with high systematic non-circular motion—the 'expanding ring of molecular clouds' 5.7.8. This feature has been modelled by an expanding ring with a radius of 190 pc, a rotational velocity of 65 km s<sup>-1</sup>, and an expansion velocity of 150 km s<sup>-1</sup> (ref. 5). The total mass of this feature is between  $10^6$  and  $10^7 M_{\odot}$ ; therefore, the energy in expansion motion may be as high as  $10^{54}$  erg. It has been suggested that the non-circular motion associated with this feature may be due to elliptical streaming in the presence of a bar9. While expanding features at large distances from the centre (such as the '3-kpc arm') may result from such elliptical streaming, it is less likely that the high non-circular motions within 200 pc of the centre have such a gravitational origin. The gravitational field in the inner few hundred parsecs is dominated by the bulge component of the Galaxy, and bulges seem to be predominantly axisymmetric systems. Kormendy<sup>10</sup> has reported that bulges in some barred galaxies are slightly elongated perpendicular to the outer bar structure, but the apparent expansion observed both in the 3-kpc arm and in the molecular ring would require pronounced elongation of the bulge in the same sense as the outer bar structure<sup>11</sup>. Therefore it is plausible that the non-circular motion of the molecular ring results from a burst of high luminosity or mass ejection from a central object.

If the ring consisted of non-interacting test particles it would

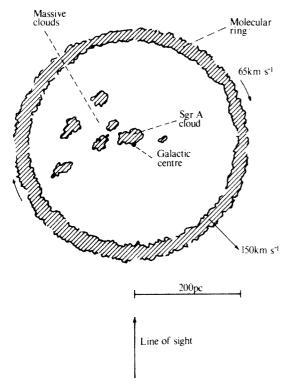


Fig. 1 The possible distribution of molecular clouds in the inner 200 pc of the Galaxy. The most massive clouds lie generally at positive longitudes and within the expanding 'molecular ring'. The Sgr A cloud may lie very close to the dynamical centre of the Galaxy.

oscillate between radii of 20 and 250 pc with a period of  $\sim 10^7$  yr. But as the ring consists of gas clouds it would not survive one such period because it would encounter gas with very different kinematics during an oscillation (that is, the massive molecular clouds closer to the centre). Therefore to maintain such high non-circular motions in the molecular cloud region (and CO line observations of the nuclei of other normal spirals do indicate the general presence of non-circular motions) a new outburst would be required every  $10^7$  yr or so, and this is the only evidence, albeit indirect, for recurring activity in our galactic nucleus. In terms of radiation from a central compact source, the centre of our Galaxy is certainly normal, but the high non-circular motions in the molecular cloud regions may be the hydrodynamic relics of short bursts of activity at the centre every  $10^7$  yr—relics which persist long after the other evidence of activity disappear.

We assume the presence of a black hole at the galactic centre with a mass  $M_h \sim 10^7 \, M_\odot$ . A compact object of this mass is entirely consistent with the velocity dispersion in the Ne II 12.8- $\mu$ M line emitting clumps in the inner 1 pc of the Galaxy<sup>13</sup> and might be identified with the milli-arcsecond radio source in this region<sup>14</sup>. There are about 10 massive molecular clouds in the inner 150 pc of the Galaxy with individual masses of the order of  $10^6 \, M_\odot$ . If we consider these 10 clouds to be particles randomly moving in a cylindrical volume ( $r = 150 \, \text{pc}$ ,  $h = 70 \, \text{pc}$ ) then a cloud will encounter the black hole on a time scale of

$$\tau \sim \frac{1}{n_{\rm c} v_{\rm c} \sigma_{\rm c}} \sim 10^7 \, \rm yr$$

where  $n_{\rm c}$  is the density of clouds,  $v_{\rm c}$  is the random cloud velocity ( $\sim 200~{\rm km~s^{-1}}$  to support the system of clouds against the galactic gravitational field) and  $\sigma_{\rm c}$  is the geometrical cross-section of a cloud determined from the observed cloud radius ( $\sim 20~{\rm pc}$ ). The clouds would also encounter one another on about the same time scale and, to maintain the random motions, the system of massive clouds would require an energy input similar to that required by the molecular ring.

A collision between a massive molecular cloud and a black hole could have spectacular consequences. The hole would accrete matter in a cylindrical column through the cloud; the radius of the column is essentially the Bondi radius:

$$r_{\rm a} = \frac{GM_{\rm h}}{v_{\rm c}^2} \sim 1 \text{ pc}$$

The fraction of the cloud mass accreted would be

$$\frac{M_{\rm acc}}{M_{\rm c}} \sim \left(\frac{r_{\rm a}}{r_{\rm c}}\right)^2 \sim 2.5 \times 10^{-3}$$

and if  $M_c \sim 10^6 M_\odot$  then the accreted mass is  $M_{\rm acc} \sim 2.5 \times 10^3 M_\odot$ .

Converting this mass to energy with an efficiency of 10% would produce a total energy of

$$E \sim 5 \times 10^{56} \text{ erg}$$

Only a few per cent of this energy need go into gas motion to account for the observed non-circular motions of the molecular clouds—both the systematic motion of the molecular ring and the random motion of the massive clouds. Assuming axisymmetric accretion and neglecting the effects of radiation pressure on the cloud, the time scale for accretion would be roughly the time during which the cloud is in contact with the hole:

$$t_{\rm a} \sim \frac{r_{\rm c}}{V_{\rm c}} \sim 10^5 \,\mathrm{yr}$$

If much of the energy came out in the form of radiation then the mean luminosity would be

$$L \sim E/t_{\rm a} \sim 10^{44} {\rm erg \ s^{-1}}$$

Therefore, every  $10^7$  yr, a cloud-black hole encounter would produce a burst of high luminosity lasting  $10^5$  yr; that is, a Seyfert nucleus would be turned on for  $\sim 1\%$  of the time.

An extremely clumpy distribution of low angular momentum gas in nuclear regions of spiral galaxies implies a highly variable accretion rate onto a massive nuclear black hole. The fraction of time in which accretion is going on is equal to the volume filling factor of the gas, and in the inner 200 pc of the Galaxy, this is a few per cent.

Hills¹ and Ozernoy¹⁵ have objected to the existence of a massive black hole in the galactic centre on the grounds that stars would be consumed too rapidly and thus produce a luminosity much higher than the observed upper limit to the luminosity of a non-stellar central point source. Assuming that the stellar density in the inner 1 pc core of the Galaxy is  $\sim 10^7 \, M_\odot \, \mathrm{pc}^{-3}$ , an upper limit of  $10^{40} \, \mathrm{erg \, s^{-1}}$  (ref. 16) on the luminosity of a compact source at the centre implies that the mass of a black hole must be less than  $10^4 \, M_\odot$ . It would be very fortuitous to see a black hole of this mass as the hole would grow to  $10^7 \, M_\odot$  in only  $10^9 \, \mathrm{yr}$ ; therefore a more probable upper limit to the black hole mass would be  $100 \, M_\odot$  (refs 1, 15).

However, these arguments only apply if the mass of the hole is substantially less than the mass of the stellar system. If the black hole has grown to  $10^7\,M_\odot$  and dominates the gravitational field in the 1-pc core of the Galaxy, then the rate of accretion of stars is very much lower again due to the slow rate of orbit diffusion in a system dominated by a point mass<sup>1</sup>. In other words, the critical radius for 'loss cone diffusion' exceeds the core radius of the nucleus. If f is the fraction of the core mass,  $M_c$ , in the black hole, then the rate at which the hole eats stars cannot exceed

$$\dot{N} = \frac{M_{\rm c}}{m_* \tau_{\rm R}} (1 - f)$$

where  $m_*$  is the mean stellar mass and  $\tau_R$  is the classical two-body relaxation time. With a 10% efficiency for converting consumed mass to energy, this implies that the luminosity due to consumption of stars is

$$L_{\rm N} = 4 \times 10^{42} \left(\frac{M_{\rm c}}{10^7 \, M_{\odot}}\right)^{1/2} \left(\frac{1 {\rm pc}}{R_{\rm c}}\right)^{3/2} (1 - f)^2 {\rm erg \, s}^{-1}$$

which is less than the observed limit if  $f \ge 0.9$ . A core mass of  $10^7 M_{\odot}$  and a stellar system mass of  $10^6 M_{\odot}$  (that is f = 0.9) are consistent with both the Ne II line observations of the core and the near-IR intensity of star light 13,18; therefore consumption of stars by such a massive black hole in our galactic nucleus does not violate observational constraints. For galactic nuclei in general, accretion of stars may have contributed to the rapid growth of nuclear black holes at earlier epochs and the higher frequency of active objects at large redshifts.

The problem with the efficiency of accretion in a cloud-hole encounter is that, due to radiation pressure on grains, the cloud may be blown away on first contact or before all the matter in the accretion column has been captured. However, because of density gradients within the cloud the captured material would probably possess some net angular momentum with respect to the hole. Therefore passage of the cloud would result in the formation of an accretion disk. In this case significant accretion and production of energy could begin after the passage of the cloud and take place on a longer time scale than that estimated above.

The probable formation of an accretion disk suggests an observational consequence for the model. As cloud-hole collisions are a stochastic process, the accretion disk axis will have no preferred orientation with respect to larger-scale galactic structure. The jets observed in radio galaxies 19 and the low-luminosity radio jets recently observed in Seyfert galaxy nuclei<sup>20</sup> are probably produced and collimated on the size scale of the accretion disk (« 1 pc), possibly along the rotation axis of the accretion disk<sup>21</sup>. Therefore, in the context of the present model, jets produced in the nuclei of spiral galaxies should have no preferred position angle with respect to the galaxy, and, from the preliminary observations, this seems to be the case<sup>20</sup>. The radio jets observed in Seyfert galaxies have linear extents of a few hundred parsecs to a few kiloparsecs; therefore, the collimating 'device' must be present and stable for time scales of at least 1,000 yr which again is consistent with the time scale of the accretion event.

Most of the energy produced in active spiral nuclei appears as electromagnetic radiation whereas the power of active elliptical nuclei goes into highly directed bulk gas motion (jets). This striking difference is probably related to the mode of accretion. Perhaps the distribution of gas in ellipticals is much less clumpy and the resulting accretion flow is smoother<sup>22</sup>. This would suggest that the position angle of elongated radio structure in ellipticals should be more correlated with the overall galactic structure<sup>23</sup> or at least with the larger-scale gas distribution<sup>24</sup>.

It would be of interest to observe the distribution and kinematics of dense gas in other spiral galaxy nuclei. In the context of this model, one would hope to observe the two species of nuclear molecular clouds in other galactic nuclei - clouds with systematic inflow or outflow and massive clouds with a low net angular momentum with respect to the galactic centre. The volume filling factor of such clouds should be of the order of a few per cent. High-resolution molecular line observations must await the development of millimetre-wave interferometers, although observations of the global kinematics of nuclear H II regions (the 'hot spot' nuclei) may also be relevant.

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## Soft X-ray imaging with a normal incidence mirror

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By sputtering<sup>1,2</sup>, it is possible to make multilayered structures (layered synthetic microstructures or LSMs) with individual layers as thin as a fraction of a nanometre. Using these techniques, we have now made a multilayered interference mirror to reflect carbon K X rays ( $\lambda = 4.48$  nm) at normal incidence. The structure consists of 76 layers of tungsten of thickness 0.765 nm with layers of carbon (thickness 1.510 nm) interspersed, deposited on a (111) silicon wafer substrate. This LSM was formed into a concave mirror of radius ~1.1 m by bending the substrate, and used in an optical set-up to form images of grids illuminated by an X-ray source. The mirror was found to have a resolution of 5 lines mm<sup>-1</sup> and an efficiency, integrated over the C K band, of between 4 and 8%.

Until now, designers of X-ray optical instruments such as telescopes and microscopes have been forced to use mirrors operating at small glancing angles  $\theta$  ( $\theta$  = angle of incidence measured from the surface tangent)3,4. This is because the refractive index  $n = 1 - \delta$  of all materials is close to unity throughout the X-ray region, so that the fraction of the radiation reflected at the interface between a homogeneous material and air (or vacuum) is very low for values of  $\theta$  much greater than the critical angle  $\theta_c = \sqrt{2\delta}$ . Nickel, for example, has  $\delta = 2.4 \times 10^{-5}$  at a wavelength  $\lambda = 0.154$  nm,  $\delta \approx 1.4 \times 10^{-2}$  at  $\lambda = 4.48$  nm, and it can be seen that the corresponding reflectance values, roughly equal to  $\delta^2/4$  for normal incidence, are extremely small.

LSMs operate in a manner exactly analogous to multilayer dielectric coatings at visible wavelengths<sup>5</sup>, and can be used to

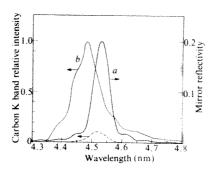


Fig. 1 Reflection of X rays by an LSM consisting of 76 layer pairs of tungsten ( $d_W = 0.765 \text{ nm}$ ) and carbon ( $d_C = 15.10 \text{ nm}$ ). Curve a (right-hand scale) is the reflectivity computed for the structure using the following optical constants: tungsten  $\delta = 1.10 \times 10^{-2}$ ,  $\beta = 1.29 \times 10^{-2}$ ; carbon  $\delta = 1.30 \times 10^{-3}$ ,  $\beta = 1.86 \times 10^{-4}$ . Curve b (left-hand scale) is the relative intensity of the C K-emission band8 The dashed curve (left scale) is the product of curves a and b. The area under this curve is 6% of that under curve b.



Fig. 2 Optical set up for normal incidence imaging with C K X rays.

increase the X-ray and UV reflectivity of surfaces. The resulting mirrors have numerous potential applications.

The simplest design is a periodic stack in which layers with a relatively high value of  $\delta$  (material A, thickness  $d_A$ ) are alternated with layers of low  $\delta$  (material B, thickness  $d_B$ ). Such a structure reflects X rays with intensity maxima at the Bragg angles  $\theta_m$  given by the relation

$$m\lambda = 2(d_A + d_B)\sin\theta_m \tag{1}$$

where m is the order of reflection. (Note that this simple relation must be corrected for the effects of refraction and absorption  $^{5.6}$ .)

We have constructed an LSM to reflect the K band of carbon ( $\lambda = 4.48 \text{ nm}$ ) in first order at normal incidence ( $\theta_1 = 90^\circ$ ), and have used it to obtain images of a grid illuminated with a carbon target X-ray tube. The structure consisted of 76 layer pairs of  $(d_{\rm W} = 0.765 \,\text{nm})$  and carbon  $(d_{\rm C} = 1.510 \,\text{nm})$ deposited on a silicon wafer with (111) surface orientation, 76.2 mm diameter and 0.38 mm thick. Figure 1 shows the reflectivity of the structure as a function of wavelength, computed using a standard multilayer program 5.6 using values of  $\delta$  and  $\beta$  (absorption index) given by Henke and Lee<sup>7</sup>. The relative intensity of the K-emission band of graphite carbon is also shown8. Although the peak reflectivity predicted for this multilayer structure is 20%, the layer period is not optimum for maximum reflectivity of C K radiation. The dashed curve in Fig. 1 is the product of the LSM reflectivity and the CK emission relative intensity, and represents an integrated reflectivity of ~6%. Note that any errors in the refractive indices will introduce uncertainties in the reflectivity calculations, affecting in particular the peak position.

The LSM-coated wafer was bent into a concave mirror of approximately spherical shape with a concentric-ring bending device patterned after that of Wassberg and Siegbahn<sup>9</sup>. This mirror was then set up on an optical bench in a vacuum chamber in the configuration shown in Fig. 2. The test object was illuminated from behind by a simple Coolidge-type X-ray tube with a colloidal graphite-coated target 1.25 mm in diameter. A special fine-grained film sensitive to soft X rays<sup>10</sup>, Eastman Kodak SO-212, was used as the detector. The film holder had a filter of 2-µm thick polycarbonate plastic foil coated with 120 nm of aluminium to exclude visible light.

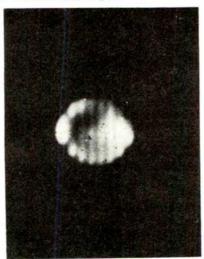


Fig. 3 Photograph of a 5 lines mm<sup>-1</sup> grid illuminated by a 1.25 mm diameter X-ray source. The non-uniformity of the image reflects the non-uniform pattern of X-ray emission by the target.

The apparatus was first aligned and focused using visible light to illuminate the test object. It was found that the silicon wafer could be bent to a radius of  $\sim 1$  m before severe aberrations set in. The object was set at a distance of 1,067 mm from the mirror and the film at a distance of 1,186 mm, with a resultant magnification of 1.11. As this off-axis configuration suffers from astigmatism, one-dimensional grids were used as targets and were set perpendicular to the plane of Fig. 2. The film was then set at position of the tangential focus.

Figure 3 is a photograph taken using C K X rays. The test object was an electroformed nickel grid with 0.1-mm bars separated by 0.1-mm spaces. The film was exposed for 1 h with

an X-ray source current of 2.5 mA at 1.5 kV.

To reduce aberrations, the mirror aperture was stopped down to 7.6 mm. With this aperture, the bright strips are easily resolved: the target period of 5 lines mm<sup>-1</sup> corresponds to about 40 arc s angular resolution. The resolution is limited by several factors, of which the most important is probably the imperfect optical figure of the mirror. A bending device of the kind used here bends even an isotropic lamina into only an approximation of a sphere, and in addition the wafer, being crystalline, tends to bend anisotropically and develop 'facets' that can be seen as a diffraction pattern in the visible light tests. Vibration of the optical bench, cantilevered in the vacuum chamber, is another possible source of degradation. Finally, the microscopic roughness of the layers themselves may cause scattering and degrade the resolution. This factor, which sets the ultimate limit of resolution that can be obtained with such a mirror, can be evaluated after the first two have been eliminated.

In this context, it is encouraging that tests with synchrotron radiation on similar LSMs yielded curves of reflectivity versus glancing angle in close agreement with those predicted from multilayer theory, indicating a high degree of layer perfection<sup>2</sup>. For the LSM described here, estimates of the reflectivity integrated over the C K band were made by densitometering an image and a piece of film exposed directly to the source behind a similar filter. The estimates lay between 4 and 8%, consistent with a 20% peak reflectivity and indicative of a close to perfect layer structure.

We conclude that normal incidence mirror elements for optical systems operating from a lower wavelength limit of 3-4 nm (soft X rays) out to 20 or 30 nm (EUV) are now feasible. Multilayer mirrors for these regions are being constructed both at Stanford University and at International Business Machines Corporation (see refs 11, 12). These optical systems have important potential applications in X-ray research and technology, and in some fields (for example, X-ray astronomy) may offer important advantages over glancing incidence optics, for example:

(1) The geometrical aberrations of normal incidence mirrors are much smaller, and the severe vignetting associated with glancing incidence mirrors is avoided thereby allowing a much larger field of view;

(2) Whereas a glancing incidence telescope reflects X rays of all wavelengths down to a certain cutoff value, the radiation reflected by an LSM is essentially monochromatic, so that auxiliary filters are not required;

(3) The collecting area of normal incidence optics is much greater for a given polished area of mirror (or substrate). Hence LSM optics, when fully developed, will probably be more cost effective.

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## Satellite measurements of H<sub>2</sub>O fluorescence in the mesosphere

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The concentration of water vapour in the mesosphere and above cannot be easily measured because the quantity of water is very small due to the low atmospheric density. Now, however, we report the observation of resonant fluorescent radiation from water vapour in the 50-95 km region by the Stratospheric and Mesospheric Sounder (SAMS) on Nimbus 7 satellite. Our preliminary data suggest that the mixing ratio of water vapour decreases with increasing height over this region with a value of  $\sim 1 \times 10^{-6}$  v/v at 75–85 km.

There are believed to be two water vapour sources in the upper atmosphere, both of which are subject to tropospheric influences. The first is direct injection by mechanisms at present poorly understood but probably involving the Hadley cell circulation in low latitudes and the 'tropopause gap' in mid-latitudes; the second is indirect injection through methane, also originating in the troposphere, which is then oxidized to carbon dioxide and water in the middle stratosphere. Evidence for the latter process is given by the apparent decrease in methane concentration at these levels and a simultaneous increase in water vapour concentration<sup>1</sup>

The predominant sinks (see reviews in refs 2 and 3) are re-exchange to the troposphere and photolysis in the upper atmosphere followed by the escape of the hydrogen produced

$$H_2O + h\nu \rightarrow OH + H$$
 (1)

Because water vapour influences upper atmosphere chemistry and hence the radiation balance and dynamics of the region, a knowledge of its concentration profile is important to our understanding of the upper atmosphere.

A multi-channel radiometer for composition sounding of the middle atmosphere, the SAMS<sup>4</sup>, was launched on the NIMBUS 7 satellite on 22 October 1978. Various channels on this instrument are designed to sense CO2, CH4, N2O, NO, CO and H<sub>2</sub>O. The instrument distinguishes radiation from a particular gas by 'pressure-modulation', an emission-sensing technique which has been described in detail elsewhere4

To increase the signal the atmosphere is viewed in the limb (Fig. 1) which has the advantage that emission observations can be made from as long a path through the atmosphere as possible against the cold radiation background of space. Such a limb path possesses an air mass about 70 times that in a vertical path above the lowest point of the path. The atmosphere's pressure profile and the geometry of the path both weight the material in the path strongly towards the tangent point giving good vertical resolution (~3 km for an infinitesimal field-of-view). In contrast, the horizontal resolution of a limb path is rather poor

The fundamental fluorescence mechanism for the 2.7-µm water vapour band is excitation by sunlight at 2.7 µm followed by radiative decay to the ground state by emission of a photon of

approximately the same frequency as that absorbed. However, competing effects can significantly influence or even dominate this simple process and of these effects three: collisional relaxation, indirect excitation and indirect radiative relaxation, need to be considered in detail. The details of the calculations will be published elsewhere.

The collisional relaxation rate of water vapour at room temperature has been measured<sup>5</sup> as  $1.1 \pm 0.4 \times 10^4$  s<sup>-1</sup> torr<sup>-1</sup> for collisions with  $O_2$ , and  $1.5 \pm 0.4 \times 10^4 \,\mathrm{s}^{-1} \,\mathrm{torr}^{-1}$  for collisions with N<sub>2</sub>. The radiative relaxation rate is 79 s<sup>-1</sup>

The unusually small ratio of the radiative to the collisional relaxation rate at one atmosphere ( $\approx 8 \times 10^{-6}$  atm compared with, for example, the  $\nu_3$  (4.3  $\mu$ m) band of CO<sub>2</sub> for which the ratio<sup>6</sup> is  $2.6 \times 10^{-4}$  atm) may be the most significant fundamental problem with this method of concentration measurement. It implies that the level at which collisional relaxation becomes important occurs very high in the atmosphere at a level of  $\sim$  90 km and the region of interest, the mesosphere, is a region of competition between collisional and radiative relaxation processes.

As the emission from the atmosphere in these conditions is almost entirely non-thermal it is necessary to compute the source function,  $J_i$ , at all levels before calculating the observed signal. Although in a general analysis account must be taken of radiative exchange within the atmosphere, in this case secondary scattering can be neglected and the source function for a planeparallel atmosphere written in the form:

$$J_{1} = n_{1}B(\tilde{\nu}_{0}, T_{1}) = \frac{\beta_{1}F_{s}}{1 + \rho_{1}} + \frac{B(\tilde{\nu}_{0}, T_{1})\rho_{1}}{1 + \rho_{1}}$$
(2)

Suffix 1 refers to the atmospheric layer,  $\tilde{\nu}_0$  is the central wavenumber of the band,  $T_1$  the kinetic temperature, B the Planck function and  $F_s$  the solar flux.  $\rho_1$  is the ratio of the collisional to radiative relaxation times, which depends on temperature and pressure.  $\beta_1$  accounts for radiative transfer within layers, absorption in the atmosphere above the layer and the structure of the 2.7- $\mu$ m band;  $n_1$  is the factor by which the source function, J, exceeds the Planck function, B. The source function shows the same spectral dependence as the Planck function at temperature  $T_1$ .  $\beta_1$  is computed from a knowledge of the spectral line distributions using the AFGL line data compilation<sup>7</sup>.

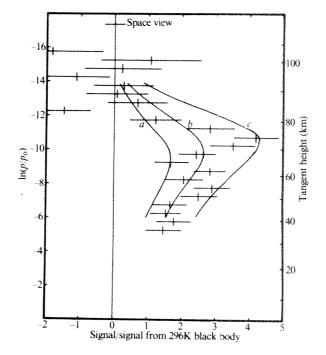


Fig. 1 The viewing geometry of the experiment. To keep the solar zenith angle to small values, data are only collected in the equatorial region. The satellite orbit height is ~950 km.

The most serious error in this equation probably arises from neglecting excitation in the 2.7-µm levels due to cascade decay from higher quantum levels, notably 101 and 011.

After computation of the source function the radiance measured by the instrument can be computed from a knowledge of the instrumental response function. For a pressure modulator radiometer this is a complicated function related to the lineshape and is non-zero only near the centres of spectral lines4. Because of this exact correlation between line positions and the instrument response function, the relative velocity of the atmosphere and the satellite must be included in the calculation. The instrument views the atmosphere in a direction perpendicular to the satellite velocity vector so that the only shift is due to the Earth's rotation. This induces a relative shift of about one doppler width in the signals and a 20% decrease in the observed signal.

Despite the enhanced emission due to fluorescence, the signals detected by the instrument are extremely noisy. This is due partly to the low density in this region and partly due to the apparently low water concentrations at these levels. Data for fluorescent measurements can only be taken when the satellite instrument is turned on, in a suitable operating mode and viewing the correct tangent height range<sup>4</sup>. The averages shown in Fig. 2 use all the available data from day 93 to day 103 1979, each point representing an average of 1,000-4,000 individual 1.8-s integrations. To eliminate effects caused by variations in the solar zenith angle, data are restricted to ±45° on either side of the equator. The vertical profile is quantized in units of 0.5 scale height ( $\approx$ 3 km). The nightside data were similarly analysed to demonstrate that this is a fluorescence phenomena and the signal in that region is zero as expected. The instrument is calibrated using the two-point method with a space view for zero signal and an internal calibration black body to provide a known radiance. The calibration procedure is more efficient than atmospheric viewing as the exact space angle is not critical and a reasonable instrument calibration can be achieved in ~36 h operating time.

The calculated signals shown in Fig. 2 have been computed from simple profiles of constant concentration with height using the data above for the collisional relaxation rate, assumed to be independent of temperature.

The most striking feature of the data is that they seem to predict a low concentration of water vapour in the upper

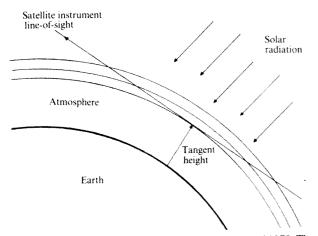


Fig. 2 Measurements and calculations for the spring of 1979. The three solid lines are calculated signals for constant volume mixing ratios of: a,  $5 \times 10^{-7}$ ; b  $1 \times 10^{-6}$ ; c,  $2 \times 10^{-6}$ . The data are shown by the crosses. The horizontal error bars are due to instrument noise and the vertical error bars to the vertical resolution of the averaging procedure. The zero radiance calibration point is established by periodically using a tangent height of >130 km, effectively eliminating all the atmosphere from the ray path. The standard deviation of the space view is shown as an isolated point at the top of the diagram. The vertical axis is  $ln(p/p_0)$  or the negative of 'scale height'. The tangent height scale on the right was constructed using an equatorial standard atmosphere for March.

mesosphere. The shape of the profile is fairly insensitive to the concentration of water vapour in the lower mesosphere and therefore the profile is reasonably consistent with either a constant, low concentration of water vapour or, more probably, a decreasing volume mixing ratio with height. Thus the shape of the profile is more consistent with that of Swider and Narcisi<sup>8</sup>, who also show a decrease with height, than that of Arnold and Krankowsky9, who show a constant mixing ratio with height.

Efforts are now being made to improve the quality of data reduction to obtain better information on the vertical distribution of water vapour as well as its seasonal variations.

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## **Bond-lengths, bond angles** and transition barrier in ice Ih by neutron scattering

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Although several studies 1.2 of ordinary ice (Ih) have established its nearly perfect oxygen arrangement and the statistical distribution of hydrogen among two symmetrically equivalent sites (the 'half-hydrogen' model3), several fundamental problems remain. The O-H bond length found in ice Ih is considerably larger than in other polymorphs of ice determined precisely4 and many arguments have been put forward against the value observed<sup>5,6</sup>. Similarly, the tetrahedral H—O—H bond angle in ice Ih differs considerably from the value observed in the vapour phase, and this led Chidambaram7 to propose a bent hydrogen bond model which further splits the atom positions, and which has not yet been verified. Finally, the mean shape of the double potential governing the atom distribution and the barrier governing the mobility still remain to be evaluated. We show here how high-precision, short-wavelength neutron diffraction data from ice Ih can be used to evaluate the molecular structure and atomic density distribution at 60 K. The unusually long -H distance is confirmed, but there is no evidence for bent hydrogen bonds. Indeed the hydrogen atom density distribution is well described by a librational motion of the hydrogen atom around the oxygen. The mean barrier height between the 'halfhydrogen' atom positions was obtained from the scattering density as 0.012 eV, which implies that the zero point motion is of high importance for the proton exchange at low temperatures.

Measurements were done at the four-circle neutron diffractometer D9 of the Institut Laue-Langevin using a wavelength of 0.7107(5) Å on a pure specimen of 6.5 mm<sup>3</sup> cut from a larger sample (supplied by A. Chaillou, Laboratoire de Glaciologie, Grenoble). The temperature was maintained using a closed loop refrigerator and data were recorded with the  $\omega$ - $2\theta$ step-scan technique. The intensities were reduced to structure factors using standard techniques8, and corrections made during the analysis for absorption9, anisotropic thermal diffuse scattering10 and anisotropic extinction11. The cell dimensions used in

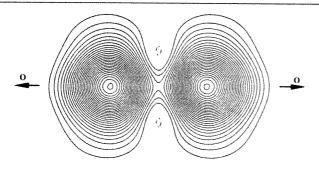


Fig. 1 Probability density map of H2 in the (0001) plane as described by harmonic and anharmonic terms up to sixth order.

the calculations are taken from the literature <sup>12</sup>: a = 4.497(1), c = 7.321(2) Å  $(1 \text{ Å} = 10^{-10} \text{ m})$ . A total of 1,887 reflections were recorded up to  $\sin\theta/\lambda = 1.07 \text{ Å}^{-1}$ , and they gave 288 reflections after averaging. The agreement factor among symmetry-related squared structure amplitudes was 0.041, and 222 of the final reflections had  $I \ge 3\sigma(I)$ .

From the data, different structural models were tested using crystallographic least-squares procedures. All the calculations were done using the Prometheus-system<sup>13</sup>, which allows incorporation and refinement of higher-order thermal tensors. The 'half-hydrogen' model was refined successfully to a weighted reliability factor  $R_{\rm w}$  of 0.013. Introduction of anharmonic thermal parameters (using a Gram-Charlier expansion of the harmonic trivariate gaussian probability function<sup>14</sup>) improved the agreement to  $R_{\rm w} = 0.012$ . To estimate the double well potential correctly from the scattering density, a calculation was carried out with the hydrogen atom located midway between the oxygen atoms, that is fixed on the saddle-point of the doublewell potential. If terms in the expansion up to the sixth order were included the refinements were successful. Only one hydrogen atom at a time was refined this way, and the two agreement factors obtained for H1 and H2 were 0.015 and 0.018, respectively. The results of all refinements show clearly the disorder of the hydrogen atoms. Figure 1 shows the atom probability density derived from the above refinements.

The results of a neutron diffraction experiment give scattering densities averaged over time and symmetry-related space in the crystal. If the molecular structure is represented conventionally by atoms undergoing harmonic motions, then observed interatomic distances must be corrected for the curvilinear relative motions of the atoms. This correction is not straightforward, as the curvilinear motion cannot be separated from the other atomic motions. However, using an anharmonic probability density function description of the hydrogen atoms allows a direct calculation of the true mode-mode distances as well as their, possibly different, equilibrium-equilibrium distances. The resulting bond lengths and bond angles given in Fig. 2 confirm the earlier observations, and indicate fundamental differences between the electronic hybridization state of ice Ih and that of

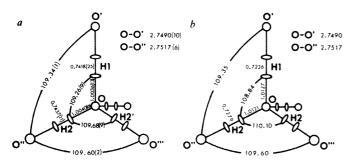


Fig. 2 a, The mean-mean bond distances and angles in the harmonic approximation. The errors (given in brackets) include the errors of the lattice constants. b, The mode-mode bond distances and angles of the anharmonic treatment. The corresponding equilibrium-equilibrium values are almost identical at a temperature of 60 K. The errors are hard to calculate and therefore not given.

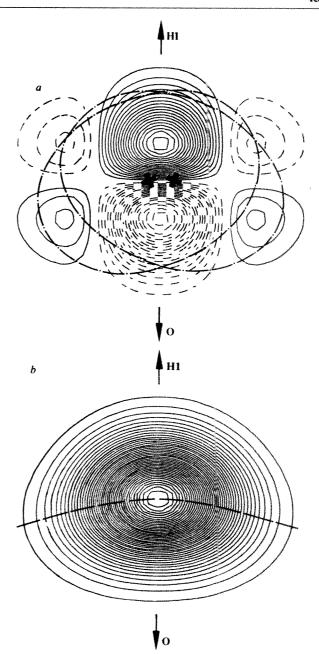


Fig. 3 a, Skewness-map of H1 in the  $(10\bar{1}0)$  plane. Solid contours show positive, dashed contours negative modifications of the harmonic (gaussian) density distribution. The dashed-dotted lines give the equiprobability contours of two atoms of the bent-bond model; their equilibrium sites (exactly in-plane) are marked by crosses. b, Total probability density map of H1 in the same (10\bar{1}0) plane as Fig. 3. The librational motion is clearly visible; the dashed line shows the trajectory of this motion. Note the similarity of this density distribution to a superposition of the bent-bond model densities given in Fig. 3a.

other ice phases studied. This could probably be caused by the complete hydrogen disorder coupled with the very low transition barrier as discussed below.

Further structural refinements were done testing the 'benthydrogen bond' model<sup>7</sup> by introducing the corresponding sixfold hydrogen splitting. The resultant agreement factors were comparable with those for the refinements of the anharmonic model. The outcome is, however, less favourable for two reasons. First, a comparison using Hamilton's *R*-factor test<sup>15</sup> and starting in both cases from the harmonic model showed the improvement to be highly significant on the 0.005 level for the anharmonic model (one parameter added), whereas the benthydrogen bond model improvements were only significant on the 0.05 level with three parameters added. Second, one would

Table 1 Root-mean-square displacements of the harmonic model

		Orientation of principal axes				
Atom	r.m.s.d. (Å)	Angle with $a_1$	Angle with $a_2$	Angle with c		
0	0.1166 (4)	90.0	90.0	0.0		
	0.1176(11)	warming.	and the same of th	90.0		
H1	0.1335 (12)	90.0	90.0	0.0		
	0.1787(8)	-	*******	90.0		
H2	0.1360(11)	90.0	36.3	111.5		
	0.1762 (11)	90.0	108.5	158.5		
	0.1801 (11)	0.0	120.0	90.0		

expect the hydrogen probability distribution to show signs of modes, or at least a positive modification in the direction of the bending of the bond, and this is not the case. Figure 3a shows the deformations from harmonicity found for one of the hydrogen atoms with the equiprobability contours of the bent-bond model superimposed. The main feature in the deformation density is clearly derived from the librational motion of the atom, whereas in the direction of the expected bending the density is merely slightly modified and as expected in the opposite way. Thus the librational motion has a slightly preferential orientation only and can be described as a motion on the surface of a sphere. A section through the total density is given in Fig. 3b. The bentbond model resembles this motion enough to mimic the density at the price of placing fractional atoms on weakly marked saddle-points in the density. The bond angle of 105° can therefore be ruled out noting, however, that the largest motion is in the 'soft' direction corresponding to the proposed bend.

The atomic root-mean square displacements (r.m.s.d.) are given in Table 1. The expected stretching frequencies calculated from the thermal motion in the harmonic approximation are  $950(38) \text{ cm}^{-1}$  for libration and  $3,780(380) \text{ cm}^{-1}$  for O-H stretch, which agree well with other experimental values10

There was some evidence for a measurable probability density between the 'half-hydrogen' positions in the Fourier maps given by Petersen and Levy1. This is confirmed by the present calculations, and clearly observable in Fig. 1. The density at the saddle-point is about four times the estimated error, and corresponds to a barrier height of ~0.012 eV for both hydrogen atoms. These low barrier heights imply both that even at low temperatures the transition frequency is high and that the zero-point motion must be of importance in the proton transfer process. From the full mean potential as derived from diffraction data one can estimate transition probabilities and lingering times, and this work is now in progress together with further studies at other temperatures and of the deuterated species.

This study confirms the earlier observations 1,2 of the geometry of the water molecule, namely that the H-O-H angle is 5° larger than in the vapour phase, and that the O-H bond is considerably larger than the vapour-value of 0.97 Å (ref. 6). Whereas it is quite common to observe increases of 2-5° in the H-O-H angle in hydrated compounds, an O-H bond length in water larger than 1.00 Å is highly unusual. As argued by Whalley6 this would correspond to a storage of elastic energy which is one-fifth of the sublimation energy or several times larger than the energy difference between ice Ih and ice II (ref. 4) or ice IX (ref. 5), which have both been observed to have mean O-H bond lengths of 0.98 Å. Likewise<sup>6</sup> the observed change in frequency for the O-H stretch does not reflect the large change in the bondlength when compared with similar results in ice II and ice IX. The present measurements of the mean structure cannot by itself be used to give an explanation of these differences, but some indications can be sought from the detailed description of the atomic density distribution (see Fig. 1). Here there is clearly a finite probability of finding H in the middle of the bond. This would correspond to a transition frequency not much smaller than the stretching frequency. In the transition state, because of cooperativity (see, for example, the ice rules3), one hydrogen atom will be approaching oxygen while another is leaving and this will create an entity with an electronic state much different from normal H<sub>2</sub>O, but undoubtedly with all O—H bonds longer than in water. The diffraction result is a sampling over all states with sampling times shorter than the transition time, and the result is an observed elongation of the O-H bond. Both ice II and ice IX are ordered structures, and one would thus, even without a detailed analysis of the scattering density, not expect a similar behaviour. Only very extensive quantum chemical calculations and lattice dynamical estimates would give the actual increase in bond length and indicate why the effective transition barrier is so low. Based on the present measurement we can only state that there seem to be coherence between the observation of the long O-H bond distances and the low barrier.

The list of structure factors is available from the authors.

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## The role of liquid halogen in the reaction of crystalline KBr with gaseous Cl2

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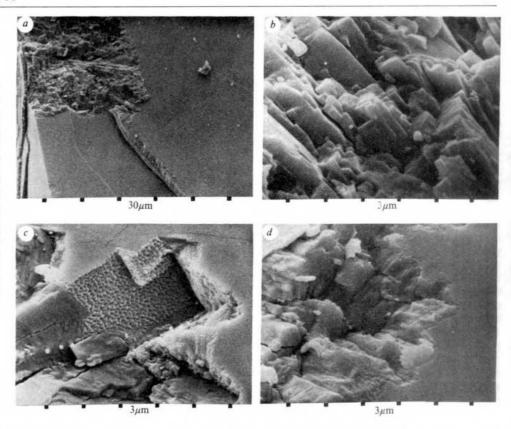
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Problems in formulating a mechanism<sup>1,2</sup> for the reaction of crystalline KBr with gaseous  $Cl_2$  (KBr(s) +  $Cl_2$ (g)  $\rightarrow$  KCl + BrCl) (a nucleation and growth process) and the role of dislocation proliferation3 in promoting interface advance have been widely discussed. The evidence that local textural changes, in the vicinity of an active reaction interface, could be observed microscopically prompted us to examine the system using scanning electron microscopy. From these textural observations together with kinetic and other measurements, we conclude that liquid halogen (Cl2, BrCl and Br2) and dissolved intermediates, contained within the pore system permeating the solid product assemblage (the nucleus), are essential participants in the chemical change. We believe that reactant KBr dissolved in condensed halogen retained in pores at the reaction interface then interacts with chlorine to form polyhalide intermediates (probably including [BrCl<sub>2</sub>]") subsequently depositing KCl product. Microscopic observations show that the nucleus is composed of KCl plates, penetrated by narrow (~0.2  $\mu m$ ) channels. This reaction model could be applicable to other systems, as previous discussions of solid state nucleation and growth processes have only rarely explicitly considered the possible participation of an intranuclear liquid and dissolved intermediates4

A feature of the reaction not emphasized in previous reports<sup>1-3</sup> was the invariable and strongly developed red-brown colouration of the product nuclei. This is important evidence of the retention of liquid bromine (and BrCl) within the product

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Fig. 1 Scanning electron micrographs of potassium bromide crystals after partial reaction in chlorine, cleaved in the (100) direction to reveal internal structures. Scales refer to spacing points at the lower edge. a, Cleavage across a nucleus; the unreacted crystal is smooth, the product is an irregular assemblage of small crystallites. Interface advance occurs in directions of principal axes. b, Texture of product within growth nuclei: KCl crystals and channels are oriented in the (111) direction (photograph edges are oriented in (100) and (010) directions). c, Details of interface after reaction at 273 K. d, Details of interface after reaction at 325 K.



assemblages which, on standing in air or vacuum at ambient temperatures, volatilized as a brown, pungent gas. The free halogen content of recently reacted salt, which we measured by titration was 3% Br<sub>2</sub> by weight, though values varied between 1 and 5% depending on reaction conditions. This quantity of liquid would occupy perhaps one-tenth of the 13% volume diminution resulting from replacement of reactant KBr with product KCl. More importantly, this volume of gaseous halogen would not be accommodated within the pore space of the residual product. Differential scanning calorimetric measurements for the freshly reacted salt, over the 310–600 K temperature range, gave only a single small broad endotherm at 350–390 K, quantitatively consistent with the evaporation of this small quantity of Br<sub>2</sub>, if present as a liquid. This is strong evidence of halogen retention during reaction.

Rate measurements were made using crystal fragments  $(0.5 \times$ 5×5 mm) freshly cleaved from a single KBr block (Specac Ltd). The vacuum apparatus used was similar to that described in ref. 5. The KBr reactant specimen was maintained at constant temperature (±0.5 K) and, after initial evacuation (10 min at 10<sup>-5</sup> torr), a known pressure (50, 100, 200 or 400 torr) of chlorine reactant was admitted. The cleaved KBr surface was observed with an optical microscope. Small nuclei were often approximately circular but after growth became square with bounding edges oriented parallel with the principal crystal axes. Predominantly diagonal cracking developed during continued growth. The red-brown colouration of the nuclei was usually most intense in the vicinity of the active reaction interface. Edge lengths of these square nuclei were measured at suitable time intervals to determine rates of interface advance. These were invariably constant-typically 0.02 µm s<sup>-1</sup> at 283 K. Rates increased with temperature by a factor of ~10 between 273 and 350 K. Growth rates for different nuclei on the same crystal face and for concurrent reactions within the same environment showed close agreement. There were, however, appreciable differences in growth rates of nuclei between successive experiments proceeding in nominally identical conditions. Rates of reaction increased with increase in the prevailing chlorine pressure. After interruption of reaction by evacuation, or a sudden reduction of chlorine pressure, the advance of the interface halted before continuation of reaction. We attribute

this to immediate volatilization followed by slow replacement of the liquid halogen within the nucleus.

Previous work<sup>1</sup> has shown that induction periods preceding the development of growth nuclei were considerable (500–5,000 min at ~290 K) as were the subsequent acceleratory nucleation processes (perhaps half as long). The equation  $(N = At^3[1-\exp{(Ct)}])$  has been used<sup>2</sup> to express the variation in the number of nuclei (N) present with time (t) (A and C are constants). We confirm the earlier observation<sup>2</sup> that nuclei are preferentially developed at sites of surface damage. We account for these kinetic characteristics by identifying potential nuclei as the finest superficial cracks within which there is condensation of halogen, BrCl and Br<sub>2</sub>, released by slow reaction of chlorine with reactant surfaces, until a liquid droplet is formed. The subsequent promotion of reaction as a growth nucleus increases the availability of bromine, so accelerating the activation of further nucleus-forming sites.

From the suggestion<sup>2</sup> that the nucleation process is catalysed by specific catalysts, we reasoned that such a substance would be a covalent liquid, capable of dissolving halogens at reaction

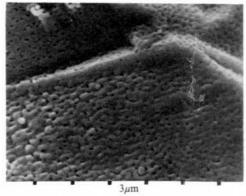


Fig. 2 Scanning electron micrograph of potassium bromide surface after cleavage followed by exposure to saturated bromine vapour for 5 min at 288 K. Cleaved surfaces were planar with step edges and exposure to bromine vapour (or liquid) caused modification including recrystallization which became more extensive after longer times of exposure.

temperature (~270-370 K) and also form a weak complex or double salt with KBr and with KCl. Tin (IV) chloride possesses these properties and a few small particles of metallic tin (20 µg) (rapidly chlorinated to SnCl4 in reaction conditions) on the reactant KBr surface reduced the induction period to nucleation at 300 K from several days to <3 h. This catalysis by liquid SnCl. is further evidence for the participation of a liquid phase in this reaction. GeCl4 and PbCl4 similarly promoted the generation of growth nuclei.

Studies using the optical microscope were supplemented using the scanning electron microscope. Surfaces only could be investigated by this technique, though information concerning internal features of partly reacted crystals was obtained through the examination of surfaces exposed by cleavage after reaction. As reacted surfaces were particularly sensitive to water vapour, atmospheric exposure was minimized. Replication<sup>7</sup> of surfaces and pore structures of developed nuclei was not possible as textural reorganization of the reactant occurred in the solvents used.

Typical internal features of growth nuclei, as revealed by cleavage, are shown in Fig. 1a-d. In Fig. 1a the featureless area of unreacted KBr contrasts with the roughened texture of the section across the assemblage of product KCl crystallites. The rectangular development of nuclei is also evident, resulting from equal rates of interface advance in the (100), (010) and (001) directions. The higher magnification in Fig. 1b shows typical rectangular crystallites of product with intercrystalline channels oriented in the (111) direction (photograph edges are aligned with the principal axes). These channels contain liquid halogen during reaction, provide access for inward diffusion of Cl2 to replace that which has reacted and permit removal of product Br<sub>2</sub> and BrCl. Characteristic textures of the irregular nucleus boundaries, the advancing reaction zone, are shown in Fig. 1c, d. There was no evidence that cracks penetrated the KBr beyond the nucleus. The etched appearance of the zone of active interfacial reaction (the centre of Fig. 1c) resembled textures developed on immersion of KBr in liquid Br<sub>2</sub> or on exposure to saturated vapour pressure of the gas; compare Fig. 1c with Fig. 2, which is a cleaved surface after exposure to saturated bromine vapour for 5 min at 288 K. Details of such surface retexturing varied with exposure times and temperatures. Retained bromine, released from nuclei on cleavage, could cause surface retexturing of adjoining zones of unreacted KBr. This again is evidence that liquid participates in the chemical change. Comparisons of Fig. 1c, d, for crystals reacted in 200 torr Cl<sub>2</sub> but at 273 and at 325 K, show that larger crystals of product were developed during the lower temperature reaction.

We conclude that the participation of a liquid phase in the interfacial processes provides a more satisfactory explanation than alternatives, such as strain, for several characteristic features of behaviour, such as the temporary cessation of interface advance after rapid gaseous reactant withdrawal, the activity of a liquid catalyst in accelerating the nucleation step and reactant textures at the interface. Furthermore, this involvement of homogeneous reactions within liquid generated in the vicinity of the interface is of wider interest because many studies have not characterized the chemical steps occurring within the reactantproduct contact zone, which are often experimentally inaccessible. Some aspects of the forms and functions of nuclei have been discussed recently<sup>5,8</sup> but more often it has been assumed<sup>4</sup> that solid products do not impede the escape of volatile products. Much recent work has related rate data to the progressive changes of interface geometry as reaction develops. The identity of a rate limiting process is sometimes inferred from the magnitude of an apparent activation energy4. The participation of a liquid localized and temporarily retained at a specialist structure within the nucleus offers an attractive explanation of the reactant species mobility that may be required to permit a bond redistribution step. Furthermore, interface processes are often accompanied by recrystallization and retexturing which similarly may proceed more readily in the presence of a liquid. The elucidation of the chemistry of interface processes is a fundamental step in identifying the factors which control the reactivities of solids.

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## Late Precambrian Keweenawan asymmetric reversals

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Palacopoles from the Keweenawan (1,200-1,000 Myr) rocks of the Lake Superior region form the western and eastern arms of the 'Great Logan Palaeomagnetic Loop'<sup>1-5</sup> (Fig. 1s). This is defined by both normal (N) and reversed (R) poles which are the consequence of at least two geomagnetic reversals<sup>4,4,7</sup>. The younger one  $(R \rightarrow N)$  has been detected throughout the Lake Superior region from various rock types. 1-4 The older reversal  $(N \to R)$  is only recorded in the lowermost Keweenawan lavas in the southern part of the lake. Wherever found, the reversals depart from the 180° symmetry (Fig. 1a)<sup>2-4</sup>. Due to these asymmetries, the Logan Loop has large segments that are devoid of data and thus the geophysical interpretations of the Loop (or 'hairpin') critically depend on the cause of the reversal asymmetry. Four models. (secondary component, apparent polar wander. Wilson's dipole offset. and non-dipole field configuration. have been put forward to explain the asymmetry. metries. Here we demonstrate that the two-dipole field configuration model can explain the reversal asymmetries and discuss the global effects of this model in the light of worldwide late Precambrian palaeomagnetic data.

First we assume that the geomagnetic field during Keweenawan time consisted of an axial geocentric dipole (main dipole,  $\dot{M}_{N,R}$ ) and an axial offset dipole ( $\dot{m}$ ) (see also refs 12–14). The geocentric dipole has two polarity states, normal and reversed (Fig. 2). The magnetic dipole moments during these states are equal  $(M_N = M_R)$  but antiparallel  $(\tilde{M}_N, \tilde{M}_R)$ . The field of the main dipole is disturbed by the persistent non-dipole (Nd)field component, the source of which is represented as the offset dipole  $(\vec{m})$ . The perturbing field represents a long-term zonal average of the Nd field  $^{0.13}$ . An asymmetric reversal will take place, when the geocentric dipole reverses while the offset dipole retains its constant polarity.

In the first case the offset dipole with normal polarity  $(\vec{m},\vec{M}_N)$ is located at the northern core-mantle boundary (Fig. 2a). If the ratio of the dipole moments (m/M) is 0.19, the inclinations at the Lake Superior area with a palaeocolatitude 53°, will be  $I_{\rm R} = -71^{\circ}$  and  $I_{\rm N} = 42^{\circ}$ , corresponding to average values observed from Middle/Lower Keweenawan data (Table 1).

In the second case (Fig. 2b), the offset dipole of reversed polarity is located at the southern core-mantle boundary. If the ratio of the dipole moments is 1.20 (that is m > M), the inclinations at the Lake Superior area with palaeocolatitude 46° will be the same as in case I. However, in this case the ratios of the global average of the Nd field to the dipole field (d) are

Table 1 Keweenawan palaeomagnetic data from the Lake Superior region (1,200-1,000 Myr)

No.	Rock types	Polarity	: U/N*	D <sub>t</sub> I (deg)	Average intensity of NRM (10 <sup>-3</sup> A m <sup>-1</sup> )	Ratio of NRM intensities $R_{\rm Int}/N_{\rm lat}$	Palacopole Lat., Long. (deg)	dp, dm (α95)† (dog)
1 <b>a</b> 1b	Duluth intrusives Duluth intrusives	N R	7/97 1/13	289, 37 91,66	2240 2440	1.09	33,192 42,200	(6.5) (7, 10)
2∎ '2b	Thunder Bay dykes Thunder Bay dykes	N R	4/47 1/17	295, 43 112, –68	5703 4120	0 72	35,181 48,213	(9.1) 7,8
3a 3b	Coldwell Complex Coldwell Complex	N R	1/7 1/13	<b>294, 54</b> 119,70			41,194 53,215	7,9 6,7
4a 4b	Upper Osler lavas Lower Osler lavas	N R	1/5 2/37	297, 40 116,61	2440 8030	3.29	34,178 46,199	6, 10 (18.4)
5a 5b	Upper Mamainee lavas Lower Mamainse lavas	N R	2/66 2/30	296, 38 115, -72	1 <b>240</b> 1 <b>28</b> 0	1.03	33,182 50,226	(16.7) (41.6)
6a 6b	Upper North Shore lavas Lower North Shore lavas	N R	3/98 3/38	290, 45 121, -63	2150 1346	0 63	32,184 50,199	(4.3) (11.0)
7∎ 7b	Upper Gargantus lavas Lower Gargantus lavas	N R	· 2/14 · 2/11	295, 39 132,69	22 5728	window.	33,183 59,213	(12.2) (59.5)
8a 8b	Lowermost South Range lavas Lowermost South Range lavas	N R	1/7 1/1 <b>4</b>	263, 37 74, -72	3789 257 <b>4</b>	0.68	10,200 29,232	8,14 7,8
	of normal data apt 8a	N N	8/350 7/3 <b>4</b> 3	290, 42 294, 42	2927‡ 275 <b>4</b> ‡	1.24 (±1.02 s.d.)	32, 187 35,185	(7.7) (4.2)
	of reversed data	R R	8/180 7/166	108, -71 115, -67	3645 3824		48,213 50,209	(8.6) (6.5)

<sup>\*</sup> U/N = number of studies/sites.

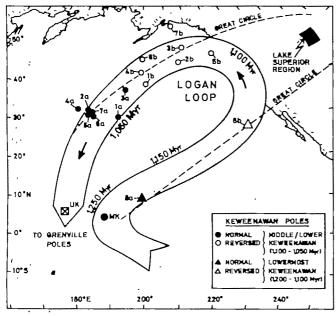
‡ Excluding 7a

unrealistically large (Nd/d = 14 (R polarity), Nd/d = 1.5 (N polarity)), whereas in case I the ratios (Nd/d = 0.56 (R polarity), Nd/d = 0.38 (N polarity)) are only about twice as high as those for the past 100 Myr (ref. 15). Note that due to axial symmetry of these models, the declinations have the observed 180° shift in both cases as observed (Table 1).

If the main dipole now makes another reversal  $(R \rightarrow N \rightarrow R)$  and so on), the inclinations will be successively asymmetric as observed in the Mamainse Point area<sup>5</sup>, provided that the offset dipole retains its polarity. Because the palaeopoles are calculated by assuming an axial geocentric dipole field, the reversed

poles (with steep negative inclinations) will be nearsided and the normal poles (with shallow positive inclinations) farsided with respect to the sampling site (Fig. 1a). The true Keweenawan palaeopoles, assuming that no polar wander has taken place across the reversal, will be the same for normal and reversed epochs and lie roughly in the middle of observed poles, 53° (case I) or 46° (case II) from the sampling site. The true poles (TKM, TKL) according to our model I are shown in Fig. 1b.

If the offset dipole (in) is of reversed polarity (case I) or of normal polarity (case II), the two models produce steep normal and shallow reversed inclinations. The distribution of



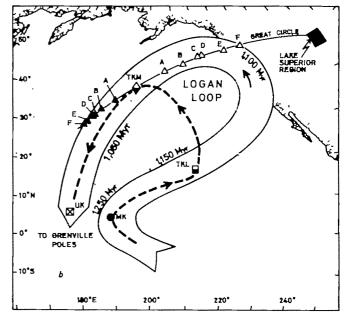


Fig. 1—a, The Great Logan Palaeomagnetic Loop as recorded in late Precambrian Koweenawan rocks from the Lake Superior region of North-America. Only such Koweenawan data, where both normal (closed symbols) and reversed (open symbols) polarities are available, are indicated. The numbers and letters refer to Table 1. The great broken circles best fit the observed poles. The swath width (10°) corresponds to an average confidence value ( $\alpha$ 95) for an individual pole determination. MK, the average pole for the Mackenzie igneous interval (1,250 Myr); UK, the average Upper Koweenawan pole (1,000 Myr). For data and references, see Table 1 and ref. 7. b. The great circles and A-Frepresent the generated APW segments produced by the two-dipole model in Fig. 2a when the dipole-moment ratio m/M values from 0.10 to 0.30 (A, 0.10; B, 0.15; D, 0.20, E, 0.25 and F, 0.30). The point C (m/M = 0.19) corresponds to the average Middle/Lower Koweenawan asymmetry with  $I_R = -71^{\circ}$ ,  $I_R = 42^{\circ}$ , respectively (Table 1). The poles TKM, (TKL) represent the true average Middle/Lower, (Lowermost) Koweenawan poles corrected for the two-dipole model. Note that there is no particular chronological order of poles from A to F m both polarities and the model allows APW 'jumps' back and forth along the generated polar wander segment. Such oscillatory APW movement has been observed in both Koweenawan<sup>5,7</sup> and coeval Grand Camyon<sup>7,26</sup> data.

<sup>†</sup> dp, dm = 95% confidence limits for the pole (single study); (a95) = 95% confidence circle for the pole (several studies combined). See ref. 7 for other refs

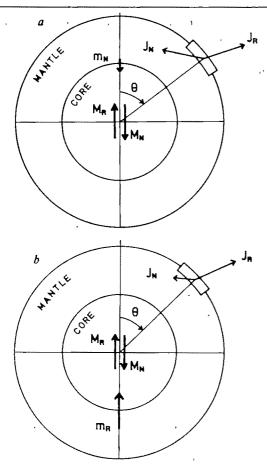


Fig. 2 Coaxial two-dipole geomagnetic field configurations which are capable to explain the late Precambrian Keweenawan asymmetries at the Lake-Superior area. a, Case I model; b, case II model (see text).

palaeomagnetic directions of NE-SW trending late Gardar dykes of Greenland 16 may be interpreted in this way.

The distributions of Keweenawan poles in Fig. 1a, fit quite well onto two great circles (western and eastern), which intersect near the sampling site. This type of pole distribution is in accordance with the axial offset dipole models 7,12. Therefore, the great depth extent of the Logan Loop may be a signature of the oscillation of the m/M ratio rather than plate motion as previously thought<sup>1-4</sup>. If we consider case I and allow the ratio m/M to vary 0.10 to 0.30 in steps of 0.05 while all the other variables remain fixed, the theoretical and purely apparent great circle polar wander segments can be generated as shown in Fig. 1b. The observed Middle/Lower Keweenawan poles (western arm) in Fig. 1a agree well with the theoretical poles. Note that the generated path segments of reversed and normal poles differ (Fig. 1b) because the function which maps the palaeomagnetic directions to the palaeopoles depends on the inclination, so that a longer segment of the palaeopoles is obtained for steeper inclinations1

In our new interpretation, the size and depth of the Logan Loop are drastically reduced. Plate motion during the Keweenawan is, however, still recognized in the new loop (Fig. 1b). The APW segments joining the Mackenzie pole (MK; 1,250 Myr) and the true Keweenawan poles (TKL, TKM) to the upper Keweenawan (UK, 1,000 Myr; ref. 7) and Grenville poles (950 Myr; ref. 18) probably represent the real paleomagnetic signature of the plate motion during the Keweenawan.

However, along the segment from the true normal pole (TKM) to the 'oscillatory' pole F (normal polarity), both plate motion and dipole oscillation are likely to cooperate in producing the APW swath. Note also that the reduced size of the Logan Loop decreases the previously proposed high APW rate.

Without the possibility of testing new models, they would be only ad hoc Nd explanations<sup>19</sup>. Three independent observations are available to test the credibility of our models.

The first test involves consideration of the distribution of the poles. The requirement that the Keweenawan poles be the consequence of the oscillation of the m/M ratio involves the assumption that normal and reversed poles lie on a great circle passing the sampling site<sup>7,14</sup>. As can be seen in Fig. 1a, this requirement is fulfilled for both arms within the limitations of accuracy of the available poles.

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The second test involves palaeointensity data. The models shown in Fig. 2 predict the palaeointensity ratios of reversed and normal rocks at the Lake Superior area to be about 0.95 (case I) and 2.3 (case II), respectively. The observed averaged ratio of NRM intensities (Table 1) for six Keweenawan formations is  $1.24\pm1.02$  (s.d.), with a range 0.63-3.29 (ref. 7). On the other hand, the average absolute palaeointensity ratio of reversed and normal units (only three formations studied) is  $1.94\pm1.64$  (s.d.) with a range from 0.62 to 3.77 (refs 7, 20, 21). Thus the palaeointensity ratio varies so widely that we cannot define its average value well and we cannot, therefore, distinguish between case I, case II and the geocentric axial dipole case. More intensity studies with Thellier method, such as performed in the Thunder Bay district<sup>7</sup>, are needed in the Lake Superior area to develop the two-dipole models.

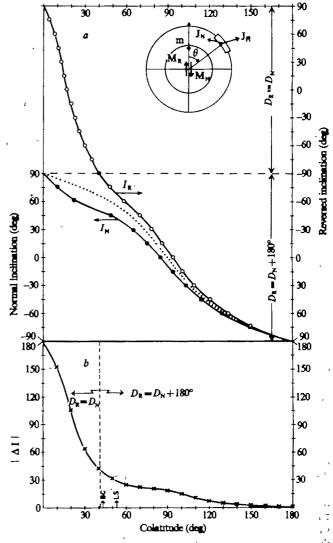


Fig. 3. a. The global values of inclinations due to the two-dipole model shown in the index figure (case I), as a function of colatitude  $\theta$ . Open (closed) symbol for reversed (normal) data. The arrows  $(I_{pe}, I_{pe})$  denote the average Keweenswan inclinations. Dotted curve is the axial geocentric dipole field inclination. b, The deviation  $|\Delta I|$  from 180° of reversals of inclinations calculated from the data shown in  $\alpha$ . The vertical broken line indicates the colatitude limit for which the declinations of normal and reversed remanences depart 180°. LS, colatitude of the Lake Superior area as measured from the true Middle/Lower Keweenswan pole (TKM in Fig. 1b), GC, corresponding colatitude of the Grand Canyon area.

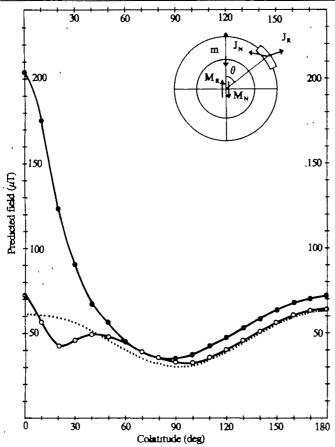


Fig. 4 The global values of field intensities due to the two-dipole model shown in index figure (case I), as a function of colatitude θ. Open (closed) symbol for reversed (normal) data. The curves are normalized so that at the palaeocolatitude 53° the intensity ratio of reversed and normal data is ~0.95 as produced by the case I model (see text) Dotted curve is the present Earth's magnetic dipole field intensity curve

The third test involves a global study of field reversals of late Precambrian age (1,200-1,000 Myr). Figures 3 and 4 show the inclination and intensity values as a function of colatitude for normal and reversed epochs predicted by the two-dipole model (case I). It can be seen (for example, Fig. 3b) that asymmetric reversals should be observed at all colatitudes, and thus the feature is not restricted to the Lake Superior region. Particularly, strong asymmetry in inclination should be observed at colatitudes  $\theta \le 60^\circ$ . Note, however, that when the colatitude is smaller than  $\sim 40^\circ$ , normal declination  $(D_N)$  becomes equal to the reversed one  $(D_R)$  (Fig. 3), and the term 'reversal' becomes confusing (see ref. 14). In case II the global asymmetries are much larger because the ratio of dipole moments is very large.

Unfortunately, the worldwide data of Keweenawan age interval reveal only few studies where both normal and reversed data are documented. These data are of poorer quality than from the Keweenawan. However, in all of these cases (for example, Barby lavas in Africa<sup>22</sup>, the Late Gardar dykes in Greenland<sup>16</sup>, the Jacobsville sandstone in Canada<sup>23</sup> and the Grenville/Sveconorwegian rocks in Canada<sup>18</sup> and Scandinavia<sup>7</sup>) the reversals are always asymmetric. The inclinational asymmetry in these studies is  $30^{\circ} \pm 18^{\circ}$  (s.d.) while the corresponding declinational asymmetry is 19°±7° (s.d.) on average. Of particular importance and in support for our two-dipole model, are palaeomagnetic data from Yenisei Ridge (1,200-900 Myr)<sup>24</sup>, where several successive reversals are found, all of which are asymmetric. Another test will become available should the reversals 25,26 in the Grand Canyon area prove to be asymmetric also, because the Grand Canyon sequence is contemporaneous with the Keweenawan one<sup>7,26</sup>. The colatitudinal difference between the Lake Superior and Grand Canyon areas (relative to true Keweenawan poles) is  $\sim 10^{\circ}$  and etherefore the asymmetry, if it exists in the Grand Canyon, should agree with the curves in Figs 3 and 4.

We conclude that the oscillating co-axial two-dipole model can describe the available palaeomagnetic data of the Lake Superior area and suggests in particular that the puzzling asymmetric reversals of Keweenawan age may reflect persistent zonal *Nd* geomagnetic field components between 1,200 and 1,000 Myr.

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## High-resolution seismic reflection profiles reveal fracture zones within a 'homogeneous' granite batholith

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Several countries are investigating the use of plutonic rock bodies for the ultimate disposal of radioactive waste material. Among criteria<sup>2,3</sup> that must be met before a rock body is mined for a repository is that it must have a low in situ permeability. As the permeability of most unfractured plutonic rocks is very low, the problem reduces to finding those bodies which do not contain major connected fractures that would allow radioactive material to be transported by water to the blosphere. We report here that a high-resolution seismic reflection survey across the central region of an Archaean granitic pluton, which on the basis of other data might be defined as relatively homogeneous, identifies major sub-horizontal fracture zones at depth. Such fracture zones, which could be a major hazard for the containment of radioactive wastes, may therefore be detected before selection of a repository site by a combination of high-resolution seismic reflection surveying and suitably located deep drilling.

The Lac du Bonnet batholith is a 96×25 km pluton trending ENE-WSW in southeastern Manitoba, close to the border with Ontario<sup>4</sup>. This pluton is one of the crystalline rock bodies being studied at four research areas in the southern Canadian Shield by Atomic Energy of Canada Limited. It contains the proposed site of Canada's Underground Research Laboratory for conducting heating and other in situ scientific experiments in the subsurface.

Geologically, the Lac du Bonnet batholith is situated within the southern portion of the English River gneissic belt, a major Archaean belt within the western Superior tectonic province of the Canadian Shield (ref. 5; fig. 1). The central and major

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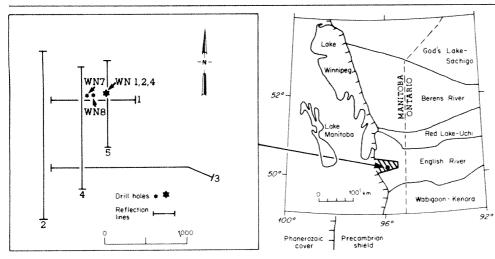


Fig. 1 Seismic survey lines across the Lac du Bonnet batholith. Inset shows the location of the surveys within the tectonic framework of the Canadian Shield<sup>5</sup>. Crosshatched region outlines the approximate location of the Lac du Bonnet batholith.

portion of the batholith has been described as a homogeneous single phase of medium- to coarse-grained granite<sup>4</sup>. Minor heterogeneity is observed in the form of xenoliths of the surrounding country rock and pegmatite and aplite dykes. Economically important mineralization (such as the Tanco deposit<sup>4</sup>) is restricted to pegmatites that intrude the country rock near the northeastern end of the batholith<sup>6</sup>.

Field evidence shows that the Lac du Bonnet batholith is intrusive into the adjacent formations and that the intrusion may have postdated the last phase of the Kenoran orogeny (~2,600 Myr) in this region<sup>6</sup>. This conclusion is supported by the fact that the batholith has undergone the least deformation of the rock units in the area<sup>4</sup>. In particular, there is only a small amount of deformation in the central region of the batholith, and this may be related to minor diapiric movement of the batholith after partial or complete crystallization.

The fractures and lineaments in the area of the batholith have been studied using satellite imagery, aerial photographs of two selected sites of 56 km<sup>2</sup> and ground observations at about 140 outcrops<sup>4</sup>. Although these studies showed three trends of fractures and lineaments (N-S, NE-SW and NW-SE), no major surface fractures were observed within the batholith. In a comparison with the lineament study by Brown and Thivierge<sup>7</sup> of 317 plutons larger than 3 km in Ontario, Tammemagi et al.<sup>4</sup> show a graph which we interpret as placing the Lac du Bonnet batholith in the top 3% of rock bodies in terms of structural integrity and percentage outcrop.

Several surface geophysical surveys have been conducted across the Lac du Bonnet batholith to delineate fractures and changes in lithology. A N-S gravity traverse across the batholith and neighbouring formations reveals the low-density batholith at the centre of a negative regional gravity anomaly. Towards the north end of the batholith, superimposed on the negative regional anomaly, is a shorter-wavelength positive anomaly that has been interpreted as due to a high-density body situated at a depth >4 km. Various density models that fit these data indicate a relatively constant density distribution within the batholith above a depth of ~4 km.

Electromagnetic and resistivity surveys 10-12 across the central portion of the batholith have failed to produce any structural

information, due to a widespread overburden layer of highly conductive clay, 0-25 m thick. Some evidence for lateral heterogeneities was obtained from a combined low-level (150-m flight altitude) magnetic total field and gradiometer survey<sup>13</sup>. The total field data show the magnetic field increasing smoothly from the boundaries of the batholith towards its centre. Near the centre, in the vicinity of the gravity high, are two particularly high-amplitude anomalies that have yet to be explained. The magnetic gradiometer survey revealed numerous anomalies across most of the batholith<sup>13</sup>, but due to the lack of outcrop it has not been possible to test either the significance of these anomalies or relate them to specific lithological or structural changes within the batholith.

In 1979 and 1980, five high-resolution MINI-SOSIE [trademark of Société Nationale Elf-Aquitaine (Production)] seismic reflection profiles were recorded within a 2.2 × 2.2 km area of the south-central region of the Lac du Bonnet batholith (Fig. 1). The principal advantage of MINI-SOSIE over conventional seismic reflection systems is its inexpensive non-destructive energy source, consisting of one or more small Earth tampers<sup>14,15</sup>. In the field, 24 receiver arrays of nine 40-Hz geophones spread over 10 or 20 m were deployed at 10 or 20 m intervals. The seismic source provided a random series of pulses to the Earth and about 3,000 pulses, suitably time shifted and added, were recorded every 10 or 20 m to provide 12-fold common reflection point coverage. A split spread configuration was adopted and a digital rate of 500 samples per s was used for each 1 s seismic record.

Conventional seismic processing techniques (static and dynamic corrections, bandpass filtering, prestack sliding gain and common reflection point stacking) have been applied to give the two E–W seismic sections shown in Figs 2 (line 1) and 3 (line 3). The line 1 section has also been passed through a wide-band velocity filter (four input traces per output trace; passband -1.7 to  $+1.7 \, \mathrm{km \, s^{-1}}$ ) to remove prominent low apparent velocity arrivals associated with the overburden layer.

Figures 2 and 3 show that the Lac du Bonnet batholith is not as homogeneous in its central region as suggested by most other studies, but contains fractures and possible intrusions. There are at least two major sub-horizontal (dips < 25°) reflection

Fig. 2 Twelve-fold common reflection point seismic section collected along line 1 (Fig. 1). Lettered reflection zones are discussed in the text. The positions of the cross lines and exploratory drill holes are shown at the top of the section. Receiver spacing 10 m. Depths shown are only approximate due to the lack of detailed seismic velocity estimates throughout the region.

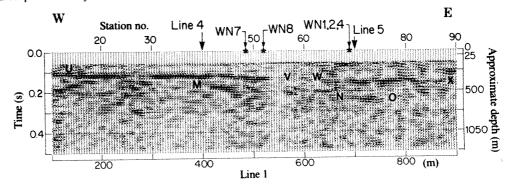
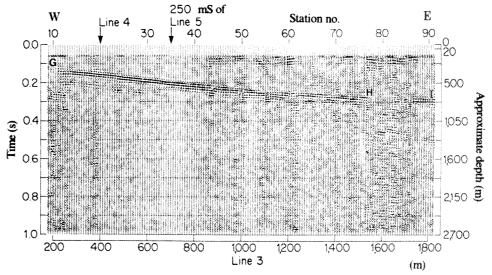


Fig. 3 Twelve-fold common reflection point seismic section collected along line 3 (Fig. 1). The position of the cross lines are shown at the top of the section. Receiver spacing was 20 m. Depths shown are only approximate due to the lack of detailed seismic velocity estimates throughout this region.



systems; UVWX in Fig. 2 and GH in Fig. 3. The true attitudes of these and other reflecting horizons are controlled by good-quality reflections on the cross-lines 1, 4 and 5 (Fig. 1).

The spectacular reflection zone GH (and possibly to I) on line 3 (Fig. 3) dips smoothly at an angle of 17° to the east and can be traced for a distance of 1.2 and possibly 1.6 km. On the N-S cross-lines this feature, which is represented by a series of strong reflections, has a more rugged character with a northward component of dip varying from 3 to 15°. It can be traced continuously from line 3 to line 1, where it is represented by the reflections MNO (Fig. 2). In the vicinity of line 1 this feature disappears and is replaced by the reflection system UVWX, which has apparent easterly and northerly dips of 8° and 9° respectively and can be mapped over the entire length of line 1 (Fig. 2).

Inclined drill holes near the cross-over of lines 1 and 5 (Fig. 1) have intersected major fracture zones starting at depths projected updip of ~360 and 600 m (ref. 16) which account for the reflections UVWX and MNO respectively in Fig. 2. The fractures near 360 m depth have been studied extensively by (1) standard geophysical logging techniques, (2) core logging (geological, geochemical and geophysical), (3) television logging<sup>16</sup>, (4) hydraulic conductivity methods<sup>17</sup> and (5) tube wave analyses<sup>18,19</sup>. The television log confirms that they dip at shallow angles in a northeasterly direction, and the hydraulic conductivity and tube wave studies show that some fractures have high permeabilities. Preliminary analysis of data from hole WN7 shows that it has intersected the fracture zone UVWX at a depth of ~300 m, in excellent agreement with the depth predicted

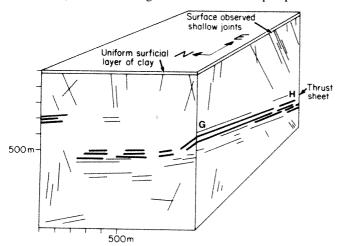


Fig. 4 Hypothetical block section of the Lac du Bonnet batholith viewed from the intersection of lines 3 and 4 (Fig. 1). A uniform (20 m thick) surficial layer of clay covers the batholith in this region. The sketch emphasizes the main features of the postulated thrust sheet G-H of Fig. 3.

from the seismic data. Note that the drilling of WN7 near this depth disturbed the water level in holes WN1 and WN4 (C. C. Davison, personal communication).

We interpret the two reflection systems as originating from a pair of en echelon fault zones with a regional dip of 12° (UVWX)-18° (GH) along an ENE-WSW to NE-SW azimuth. Locally, the attitudes of the faults vary due to relief along the strike directions (Fig. 4). The projected surface outcrops of the fault zones lie beneath the Winnipeg River, and may have influenced the drainage pattern in this region. We suggest the original fractures developed with some relief along strike and movement was along the smooth shear planes (UVWX and GH in Figs 2 and 3 respectively) parallel to the dip direction.

The low dip angles of the fault zones are probably indicative of thrust faulting, caused by horizontal compressional stress in an ENE-WSW to NE-SW direction, approximately parallel to the long axis of the batholith. This stress field was not prevalent in Archaean times, during the batholith's intrusion<sup>5</sup>, when the largely N-S compression of the regional stress field resulted in the E-W trending tectonic belts of the western Superior province (Fig. 1). However, the postulated ENE-WSW to NE-SW compressional stress field is parallel or sub-parallel to the current regional stress field inferred for much of northern North America. Focal mechanism studies of earthquakes in eastern Canada and the eastern United States show E-W to NE-SW deviatoric compressional and near-vertical deviatoric extensional axes<sup>20,21</sup>, and this same stress field has been determined by hydro-fracture measurements<sup>22</sup> and with greater variability by in situ stress measurements at Red Lake, 120 km north-east of the Lac du Bonnet batholith, show predominant NE-SW deviatoric compression<sup>24,25</sup>. A similar NE-SW stress field has been suggested for sediments beneath the western Prairies of Canada, based on the preferred orientation of spalling in oil wells and the results of hydraulic and steam fracturing<sup>26</sup>. Although the E-W to NE-SW stress field may be a feature of much of the northern North American continent, the source of the stress may not be the same for the different regions.

This study of the Lac du Bonnet batholith, a rock body which on the basis of other data may be classified as relatively homogeneous, shows it to contain numerous fractures, some of which are interconnected and may reach to the surface. A hypothetical block section that might be viewed from the junction of lines 3 and 4 in Fig. 1 is sketched in Fig. 4. The high permeability and size of some of these fracture zones (UVWX is >50m thick and affects an area of at least  $400\times800$  m) demonstrate that they may be a major problem in the search for a suitable repository site.

Our results indicate the feasibility of subjecting any potential repository site to a comprehensive high-resolution seismic reflection survey. A three-dimensional seismic reflection survey, combined with an appropriate exploratory drilling programme,

could delineate blocks of a rock body that may be relatively unfractured and thus suitable for a nuclear waste repository.

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## **Bacterial origin of East Australian** continental margin phosphorites

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Many models of the origin of marine phosphorites require a sediment rich in organic matter, which by decomposition releases phosphorus for the precipitation of carbonate fluorapatite1-7. This concept is useful in explaining contemporary phosphorite in areas of very high productivity off the coasts of Peru-Chile and South West Africa 8-10 where there are high organic matter fluxes to the sea floor<sup>4-7,11</sup>. It does not explain the origin of marine phosphorite deposits which formed in regions of limited oceanic upwelling and productivity 12-1 The East Australian continental margin, an area of phosphomodern analogue of an 'East Coast' phosphogenic province 15-17 with low productivity over the uncertainty genesis throughout the late Pleistocene and Holocene 13,14 with low productivity over the upper slope region where the most recent phosphorites have been found<sup>18</sup>. Evidence is reported here for the carbonate fluorapatite in the East Australian phosphorites being located within bacterial cellular structures, and a model proposed for the origin of these deposits through the slow bacterial assimilation of phosphorus from seawater in an area of restricted sedimentation.

Phosphatic nodules and sediments were recovered by dredging and piston and box coring on the East Australian continental shelf and upper slope between 29° and 31° S at depths ranging from 210 to 450 m. In box cores, late Pleistocene to Holocene phosphatic nodules occur within unconsolidated sediments of silt to sand-sized quartz, glauconite pellets and planktonic foraminiferal tests. The sediments have a grain-supported fabric: the low concentration of fines is believed to have resulted from winnowing processes. Nodules of Holocene age (from uranium-series data) always occur within a few centimetres of the sediment surface. Nodules having uranium-series ages in excess of 240,000 yr often occur within the top 10 cm of the cores. This is consistent with a low sedimentation rate, or with significant mechanical reworking and erosion. Based on the uranium-series dating of three phosphatic nodules occurring at different depths in piston core P904 (Table 1), we interpret the distribution of nodules in the uppermost parts of the cores to be the result of a low sedimentation rate in the region. Assuming that the nodules formed at or near the sediment-water interface, the uranium-series ages indicate an average sedimentation rate of <1 cm kyr<sup>-1</sup>. Table 2 summarizes geochemical data from six samples of nodules and sediments. The organic carbon concentrations are low compared with values reported for high productivity phosphogenic provinces. The phosphorus values for the sediments are also low. This suggests that carbon and phosphorus fluxes to the East Australian sediments are several orders of magnitude lower than those obtained from Peru-Chile and South West Africa<sup>4,5,11</sup>

Examination of freshly fractured surfaces of late Pleistocene to Holocene phosphatic nodules by scanning electron microscopy (SEM) with energy dispersive analysis revealed that the detectable phosphorus is located in structures morphologically resembling bacteria. The cellular structures are composed of apatite and resemble non-filamentous, botuliform bacilli 1.5-2.0 µm in length (occasionally as short as 1.0 µm) and 0.75-1.0 µm in width, generally cylindrical, but commonly with a tendency to be ovoid to subturbinate, with one end tapering to become more pointed than the other (Fig. 1a).

Together with fine clay minerals, the bacilli are concentrated in the matrix of the nodules, and occur attached to all solid surfaces including quartz grains, glauconite pellets and foraminiferal tests. Attachment of the cells to a surface is generally by the narrow end of the cell, but there is no development of a stalk. The morphology of the bacteria is seen most clearly on shell fragments where their numbers are small (Fig. 1b, c). There the bacilli did not form chains or mass into colonies, but appear to be randomly distributed, as if a motile cell had migrated to an anchorage. Where aggregations became greater, contiguous colonial growth occurs (Fig. 2a), and in some instances, dense bacterial colonies are layered with the long axes of the cells parallel to one another but normal to the supporting surface (Fig. 2b).

In contrast to the phosphatic nodules, the associated sediments are almost free of bacterial structures. Pyrite framboids are present in both the late Pleistocene to Holocene nodules and in the sediments in association with carbonaceous material; but they are not abundant, and where present usually occur within foraminiferal tests. Middle Miocene ferruginous phosphatic nodules from the same region contain few bacilli: the apatite is mostly present as well-developed crystals (Fig. 2c). In both the late Pleistocene to Holocene and middle Miocene nodules, sharp, well-defined X-ray diffraction peaks indicate good apatite crystallinity.

Given that all of the carbonate fluorapatite in the younger East Australian phosphatic nodules is present within bacterial structures, what evidence is available to help define the environmental conditions suitable for their proliferation, and identify their type? A consistent morphological feature shown in our micrographs is the relatively uniform size and shape of the bacilli, both within and between sampled sites. This suggests that we may be dealing with one specific organism, rather than with the range of forms that would signify a mixed population.

The absence of a mixed bacterial population indicates that the ecological niche occupied by this organism was highly restrictive, and possibly unique to a single species or group of closely related and morphologically indistinguishable species. The high degree of preservation of whole bacterial cells in the nodules suggests that they have been free from competition, predation and grazing. The most likely synopsis of these ecological condi-

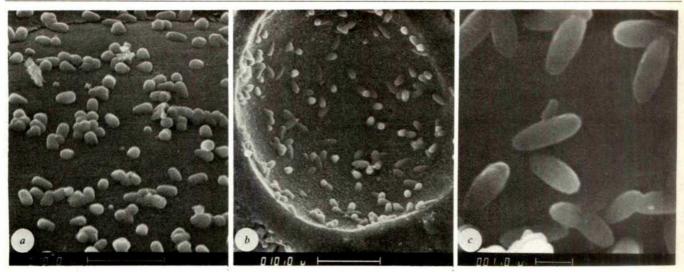


Fig. 1 a, Bacterial cells replaced by apatite encrusting a foraminiferal shell surface. Amongst the many sausage-shaped bacilli are some that are turbinate and taper towards one end. Early Pleistocene phosphorite nodule P895(120cm) – 1. Scale bar, 10μm. b, The scatter of bacterial cells in this foraminifer suggests that the bacilli may have been motile and migrated to this protected site before anchoring by the narrow end. Early Pleistocene phosphorite nodule P884(78cm) – 1. Scale bar, 10μm. c, The characteristic bacillary shape, now replaced by apatite, illustrates the typical morphology of the bacteria found in the East Australian phosphorite nodules. Holocene phosphorite nodule P883(20cm) – 1. The bacteria occur on a shell surface. Scale bar, 1μm.

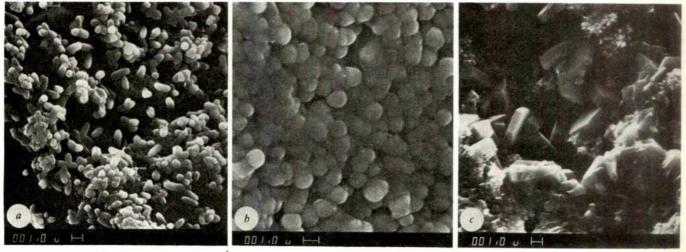


Fig. 2 a, In sites where bacterial growth became intensive enough to form colonies, the uniformity of cell shape and the state of their preservation are outstanding features. Holocene phosphorite nodule P883(20cm) – 1. Bacteria are clustered on detrital silicate grain. Scale bar, 1μm. b, In tightly-packed colonial growth the bacterial cells are polarized with long axes normal to the supporting surface, but apatite replacement still reveals confinement within cellular structures. Early Pleistocene phosphorite nodule P895(120cm) – 1. Scale bar, 1μm. c, Well crystallized apatite filling fracture within middle Miocene phosphorite nodule G18. Scale bar, 1μm.

tions suggests a biological desert in which few organisms grow, in which the growth rate of the individual cells may be quite slow, and in which a slow sedimentation rate provides minimal energy and/or nutrient inputs.

While there are limited criteria on which to base the identification of the bacteria, there are some morphological features which preclude membership of several taxonomic groups commonly implicated in phosphate transformations. On this basis, the sheathed bacteria which metabolize iron, the budding bacteria which metabolize manganese, and the stalked bacteria such as the caulobacters<sup>19,20</sup>, which grow in very dilute media, are excluded. Similarly they are not slimy filamentous actinomycetes such as *Bacterionema matruchotii* or *Leptotrichia buccalis* which are found in the oral cavities of man and primates<sup>21,22</sup>, and which deposit apatite in dental calculus and plaque deposits. The cells are not curved in the manner of spirillums or vibrios; nor flexuous, sinusoidal, nor coccal. None of the micrographs display swellings implying Gram-positive sporing rods, or the cluster formations of the corynebacteria<sup>23</sup>.

About 90% of the bacteria isolated from marine environments are Gram-negative rods; usually pseudomonads predominate<sup>24,25</sup>. Many of these bacteria are not specific to marine

environments, but can occur in fresh waters and soils<sup>26</sup>. The bacteria that we have observed are probably Gram-negative aerobic pseudomonads or facultatively anaerobic enterobacteria. The most attractive hypothesis is that they are facultatively chemolithotrophic pseudomonads with the ability to utilize hydrogen or carbon dioxide. This would have enabled them to inhabit the restricted ecological niche with very low energy input which the East Australian upper continental slope provided during phosphogenesis. A parallel group of halophobic pseudomonads inhabit reticulated distilled water storage systems where both nutrient and energy supply are drastically limiting<sup>19</sup>.

The phosphorus contents of microorganisms are usually high, of the order of 5-10% of the organic fraction<sup>27</sup>. Where an organism grows very slowly and is efficient in extracting phosphorus from the low concentrations commonly present in seawater, it may accumulate normal or even elevated concentrations of nucleic acids, phospholipids and polyphosphate. The apatite forming the cell outline is not an encapsulation, but rather an intracellular deposit. It is not likely to have precipitated internally in vivo, but is more likely a post mortem transformation of assimilated phosphorus. This presupposes either that the bacteria retained cellular organization for an

P852

P895

P906

P887

G19

Table 1 Uranium-series isotope and age data for phosphatic nodules and calculated sedimentation rates for piston core P904 (29°18.0' S, 153°50.3' E, water depth, 405 m)

Depth of nodule from surface (cm)	P <sub>2</sub> O <sub>5</sub> (%)	U (p.p.m.)	Th (p.p.m.)	$^{234}\mathrm{U}/^{238}\mathrm{U}$	$^{230}$ Th $/^{234}$ U	<sup>230</sup> Th/ <sup>232</sup> Th	Age(kyr)	Age(kyr) <sup>230</sup> Th <sub>c</sub> *	Mean sedimentation rate for stated interval (cm kyr <sup>-1</sup> )
35	7.9	91	3.5	$1.13 \pm 0.013$	$0.49 \pm 0.018$	43±7	$<72\pm4$ $<181^{+13}_{-9}$ $>240$	72	0-35 cm: 0.49
122	9.5	126	4.7	$1.07 \pm 0.014$	$0.83 \pm 0.021$	71±9		177	35-122 cm: 0.83
146	7.2	59	5.3	$1.09 \pm 0.014$	$0.91 \pm 0.020$	33±3		>240	122-146 cm: <0.38

153°19'

30°41

Site and geochemical data for east Australian continental margin phosphatic nodules and sediments Table 2 Corg (%) P<sub>2</sub>O<sub>5</sub> Fe<sub>2</sub>O<sub>2</sub> Water depth Depth in Age (kyr) Long. (m) (%)(%)Description core (cm) Lat. Sample no. 450 0.57 0.29 3.58 3.5 - 5.031°01.1' 153°18.7' 153°49.3' 390 0.49 0.20 4.10 125 29°23.5 Sediment 22°20.8 153°50.0' 415 0.51 0.36 5.27 82 4.36 22\* 153°50′ 370 0.68 7.5 29°18.8 0 - 3Phosphatic 194\* 29°23' 153°50′ 385 0.43 15.1 5.17 G7-13

230

nodule

appreciable period after active metabolism ceased without degeneration by lysis and without leaching losses of soluble polyphosphates, or that precipitation of apatite occurred rapidly.

There is no evidence to support a hypothesis that the growth of the organism we have identified in the East Australian phosphorites needed episodic accessions of readily decomposible organic detritus. In particular, there is a lack of appreciable sulphides, an absence of fish bones in the sediments, uniformity of the bacterial population, and very low organic carbon concentrations in both the sediments and the phosphatic nodules.

While limited seasonal upwellings (and associated higher nutrient levels) are known to occur sporadically on the East Australian continental shelf<sup>28,29</sup>, these usually occur within 15 km of the coast and are unrelated to the phosphogenic region, which is some 35-40 km offshore.

We conclude that the late Pleistocene to Holocene phosphatic nodules on the East Australian continental margin have been formed by the accumulation of carbonate fluorapatite during the post mortem alteration of phosphorus-rich bacterial cells. Assimilation of high phosphorus levels from seawater of average phosphorus concentration would occur slowly in a slow-growing bacterial population. For this reason a very low sedimentation rate is critical, otherwise the bacterial growth would be swamped by sediment. We envisage the microbial growth gradually cementing the unconsolidated sediments30, although fine clay minerals in the phosphatic sediment must also be important in the lithification process. The cemented phosphatic layer is later broken into nodules, either by the action of endolithic organisms or by the effect of strong bottom currents, or probably both. The presence of fine clay within the bacteriarich matrix suggests that bottom current velocities during times of phosphogenesis were substantially lower than present day current velocities<sup>31,32</sup> that are sufficiently high to winnow fines from the sediment. We are not certain of the environmental factors which controlled the onset and termination of bacterial growth and phosphogenesis, although they may have been related to a subtle balance between erosion and deposition which was controlled by variations of the East Australian current.

Carbonate fluorapatite in the middle Miocene nodules occurs largely as well-developed crystals. These may have formed during diagenesis (Fig. 2c) of phosphate present initially in bacterial structures. Some similarities of depositional environment of the East Australian phosphorites to other more ancient deposits of the 'East Coast' type may suggest that some of these older phosphorites also had a bacterial origin, but that this has been obscured by subsequent diagenetic processes. Structures composed of apatite and similar to those we have described also occur in phosphorites off Peru-Chile and South West Africa<sup>6,33</sup> If they prove to be bacterial, our model for phosphorite formation may represent a general phenomenon.

8.4

43.12

Middle Miocene†

Most samples were collected in 1979 from the vessel RV Tangoroa through the courtesy of Dr D. Cullen of the New Zealand Oceanographic Institute. Professor C. C. von der Borch provided samples from locations G7, G18 and G19. Dr R. C. Foster provided TEM data during early stages of the work and with Drs A. Pearce, F. S. Godfrey and B. Scott gave helpful suggestions. SEM work was carried out at the University of Adelaide Electron Optical Centre. Financial support was provided by the Australian Research Grants Committee.

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0.14

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Errors quoted based on counting statistics  $(\pm 1\sigma)$ . \* Corrected for common Th using  $^{230}$ Th<sub>c</sub> =  $^{230}$ Th<sub>m</sub> –  $(1.5 \times ^{232}$ Th<sub>exp</sub> $(-\lambda_{230})t)$ , where  $^{230}$ Th<sub>m</sub> is the measured activity of  $^{230}$ Th.

<sup>\*</sup> Uranium-series data. † Palaeontological data.

# Atmospheric concentration of ammonia in Massachusetts and deposition on vegetation

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Ammonia has an important role in atmospheric chemistry and the soil nitrogen cycle, and it has long been suggested that terrestrial ecosystems may be important sources and sinks for atmospheric ammonia in both gaseous and particulate form<sup>1</sup> But there is relatively little information on the average atmospheric concentrations of NH<sub>3</sub> gas and particulate NH<sub>4</sub> and their fluxes to and from ecosystems. Furthermore, much existing data are unreliable because of difficulties in separating gaseous NH<sub>3</sub> from particulate NH<sub>4</sub> (refs 1, 5). We have used a new method<sup>5,6</sup> which overcomes these difficulties and have made continuous measurements for a 1-yr period at a rural location in Massachusetts. From these data and from measurements of the rate of deposition of ammonium on plastic and natural leaves, we conclude that particulate NH<sub>4</sub> can be a significant nitrogen source for ecosystems, especially pine forests. In contrast, gaseous NH3 is probably of little importance as its concentration is very low.

In our measurements (Fig. 1) we found a strong seasonal pattern in the atmospheric concentrations of NH<sub>3</sub> and NH<sub>4</sub>, with maximum values during the summer months. This suggests that much of the NH3 may come from agricultural or natural sources, rather than from the combustion of oil and coal. The measured concentrations of particulate NH<sub>4</sub> are similar to those reported elsewhere in the United States but the values for gaseous NH<sub>3</sub> are lower than most other reports<sup>1,3</sup>. The reasons for this are unknown but may be partly due to methodology. Most measurements of NH<sub>3</sub> have removed NH<sub>4</sub> particulates by placing a filter upstream of the NH<sub>3</sub> collector. Reactions on the filter may release NH<sub>3</sub>, causing an overestimate of NH<sub>3</sub> concentration<sup>1,5</sup>. It is unlikely that the alternative methodology that we used underestimated gaseous NH<sub>3</sub>, as placing collection tubes in series demonstrated a collection efficiency of at least 90%, which is consistent with theoretical prediction<sup>5,6</sup>. Another factor may be the high concentrations of acidic aerosols in the eastern United States. Calculations based on NH<sub>4</sub> and H<sup>+</sup> concentrations in precipitation predict7 very low concentrations of atmospheric NH<sub>3</sub>. Finally, there may be few major sources of atmospheric ammonia in the region, due to the acidity of the soil and low density of farms. Using similar methods, low concentrations (0.2 µg m<sup>-3</sup>) of gaseous NH<sub>3</sub> have also been measured in Sweden<sup>5</sup>, but higher values (3 µg m<sup>-3</sup>) in the Netherlands<sup>6</sup>.

Farquhar et al.<sup>2</sup> have determined the NH<sub>3</sub> compensation point, at which leaves neither gain nor lose NH<sub>3</sub>, for several plant species. Extrapolation of their data to our mean summer temperature of 20 °C suggests a NH<sub>3</sub> compensation point of 1  $\mu$ g m<sup>-3</sup>, which is much higher than our mid-summer NH<sub>3</sub> concentration of 0.2  $\mu$ g m<sup>-3</sup>. This suggests that Massachusetts ecosystems might actually lose NH<sub>3</sub> to the atmosphere. Even if NH<sub>3</sub> uptake is possible at these low concentrations, measurements of uptake at higher concentrations suggest a deposition velocity of ~1 cm s<sup>-1</sup> (ref. 2). Assuming leaves to be present from 1 June to 1 October, this deposition velocity results in an estimated NH<sub>3</sub> uptake equivalent to 0.13 kg N ha<sup>-1</sup>, which is negligible compared with the nitrogen input in precipitation.

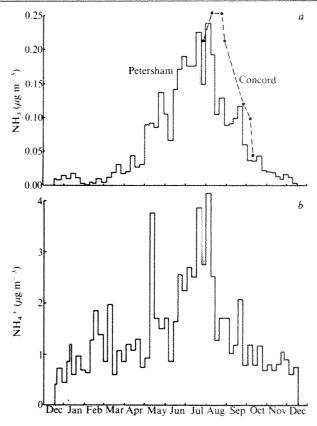


Fig. 1 Atmospheric concentrations of NH<sub>3</sub> (a) and NH<sub>4</sub><sup>+</sup> (b) measured during 1980 in a forest clearing at Petersham in central Massachusetts. Gaseous NH3 was collected in glass tubing coated for 45 cm with 5% oxalic acid as described by Ferm<sup>5</sup>. The intake was at 1.5 m height and air was drawn through the tubing at a flow rate of 2.71 min<sup>-1</sup>, using a vacuum pump and critical orifice to , using a vacuum pump and critical orifice to control flow rate. Collections were made over 7 days with particulate NH<sub>4</sub> being collected at the downstream end of the tubing on a 0.4 μm, 47 mm, Nucleopore filter. Analysis of NH<sub>3</sub> was by electrode (Orion 95-10). The means of two collectors are plotted; the average difference from the mean of the individual values was 8.6% for NH<sub>3</sub> and 3.2% for NH<sub>4</sub>. Also shown are values for NH<sub>3</sub> concentration (mean of two collectors) measured over 24-h periods during late summer 1980 at Thoreau's Bog, Concord, near Boston; the intake height was 0.8 m and analysis of NH3 was by the phenol-hypochlorite method.

Unlike gaseous NH<sub>3</sub>, particulate NH<sub>4</sub> may be a significant nitrogen source for forests in Massachusetts. However, available data do not allow an accurate estimate of the rate of deposition of particulate NH<sub>4</sub> to forests, so we made experimental determinations. A correlation coefficient of 0.67 was found between the NH<sub>4</sub> collected on rectangular plastic leaves and the concentration of particulate NH<sub>4</sub> in the atmosphere (Fig. 2). Other factors such as wind speed and particle size may also have influenced the rate of deposition, and there may have been some exchange of gaseous NH3 between the atmosphere and the deposited aerosols. In the above experiments leaves were exposed at a height of 11 m in a clearing, but in other experiments (to be described elsewhere) there was no difference in NH<sub>4</sub> deposition on leaves placed in the canopy at two different forested sites. Likewise, there was no difference in NH4 deposition on a surface area basis between natural leaves of Kalmia latifolia and Pinus strobus and plastic leaves simulating broad leaves and pine needles. Unexposed leaves were used as blanks to control for NH<sub>4</sub> arising from internal leaching. However, the rate of NH<sub>4</sub> deposition per unit of surface area was 2.0 times greater (r = 0.93, P < 0.01) for plastic leaves that simulated Pinus strobus needles than for rectangular plastic leaves.

Our measurements of the atmospheric concentration of particulate NH<sub>4</sub> and its rate of deposition on plastic and natural leaves can be combined to estimate the annual nitrogen input to

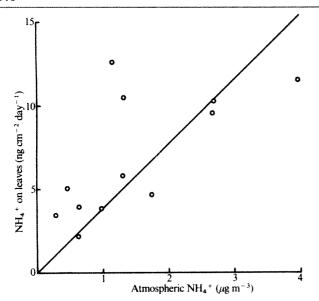


Fig. 2 Deposition of NH<sub>4</sub> on plastic leaves as a function of atmospheric concentration of particulate NH<sub>4</sub><sup>+</sup>. The leaves were 10 rectangles of polyethylene  $(0.1 \times 2.0 \times 5.8 \text{ cm})$  that were exposed at a height of 11 m for 24 or 48 h. Ammonium was leached from the leaves by immersing each one in a vial containing 20 ml of deionized water for at least 1 min with agitation. Ammonium was measured by electrode, with a correction for unexposed blank leaves. Each point is the mean of the 10 individual determinations; the standard error averaged 12% of the mean. A linear regression through the origin is plotted: r = 0.67, P < 0.02

forests from this source. Assuming a leaf area (both sides) of 8 m<sup>2</sup> per m<sup>2</sup> of ground surface and using the regression line in Fig. 2 we calculated a deposition velocity of  $0.35 \text{ cm s}^{-1}$  for broad-leaved trees. The calculated deposition velocity for pines was 0.72 cm s<sup>-1</sup> because deposition of NH<sub>4</sub> per unit of surface area was 2.0 times greater (see above). For deciduous, broadleaved forests the leaves were assumed to be in place from 1 June to 1 October, during which time the average particulate NH<sub>4</sub><sup>+</sup> concentration was 2.1 μg m<sup>-3</sup>. For this period the calculated deposition of NH<sub>4</sub><sup>+</sup> was 0.6 kg N ha<sup>-1</sup>. For pine forests the leaves were present all year and the average concentration of  $NH_4^+$  was 1.4  $\mu g m^{-3}$ . From this we calculate a  $NH_4^+$  deposition of 2.5  $kg N ha^{-1} yr^{-1}$ .

The above estimates suggest that dry deposition of particulate NH<sub>4</sub> on leaf surfaces may be a significant sink for atmospheric ammonia and a source of nitrogen for forests, especially pine forests. There is little nitrogen fixation in Massachusetts forests8, so the only known nitrogen input is from precipitation which totals  $6.5 \text{ kg N ha}^{-1} \text{ yr}^{-1} \text{ as NH}_4^+ \text{ plus NO}_3^- \text{ at a location } 160 \text{ km}$  to the north. Thus dry deposition of particulate NH $_4^+$  appears to be a significant source of nitrogen relative to precipitation, while dry deposition of gaseous NH3 is not. This supports previous evidence that dry deposition of other components of aerosols such as acids, nitrates and sulphates is important<sup>10-14</sup>. Further studies are needed to quantify these inputs more precisely, but it seems likely that dry deposition of aerosols containing ammonium and nitrate is an important factor in maintaining the productivity of pine and other forests. This may be especially true for forests on poor soils of low nitrogen content.

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## A sterile defender morph in a polyembryonic hymenopterous parasite

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Early in the development of polyembryonic Encyrtidae, a number of larvae are formed that are morphologically different from their later sibs. These larvae die without pupating. They have been described as asexual<sup>1</sup>, teratoid<sup>2,3</sup> or precocious<sup>3</sup>, and it has been suggested that they render the host suitable as food and habitation for the later, normal larvae1.4. I now report findings which demonstrate that the precocious larvae constitute a defender morph, eliminating other internal parasites that would otherwise compete with, and potentially preclude the development of, their normal sibs.

A colony of an as yet undescribed species of the encrytid genus Pentalitomastix has been maintained in the laboratory on its natural host, the phycitid moth Anagasta kuehniella (Zeller). Female wasps oviposit in the host egg but the resulting parasitized host larva does not succumb until after it spins a cocoon some 6 weeks later. Instead of pupating, the host larva 'mummifies', becoming no more than a sac filled with 100-200 parasite pupae. Sequential dissections of 250 parasitized hosts reveal that as in other polyembryonic encyrtids 1,2,5, the parasite egg develops into a mass of apparently undifferentiated cells, the polygerm, which gives rise to the numerous larvae that fill up the host 'mummy'. The first one or two parasitic larvae are invariably precocious (Fig. 1) and are found in the host larva within 10 days after deposition of the parasite egg. Precocious larvae continue to appear as the polygerm increases in size (24 have been found in one host) until the stage,  $4\frac{1}{2}$  weeks after egg deposition, when the polygerm dissociates into normal larvae. By the sixth week, the normal larvae are mature (Fig. 2) and only dead precocious larvae are recovered from dissected hosts.

Two solitary endoparasitic wasps, the braconid egg-larval parasite Phanerotoma flavitestacea Fischer, and the ichneumonid larval parasite Trathala sp., have also been maintained in the laboratory with Anagasta kuehniella as a factitious host. These parasites were originally reared from the phycitid Ectomyelois ceratoniae (Zeller). Dissections of 33 multiply parasitized A. kuehniella larvae exposed as eggs to P. flavites-

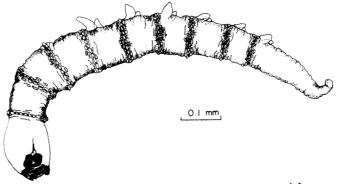


Fig. 1 Precocious larva of Pentalitomastix sp. recovered from 14-day-old A. kuehniella larva. The number of paired dorsal papillae is variable.

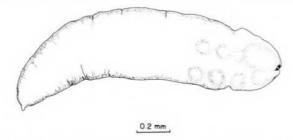


Fig. 2 One of 164 mature normal larvae of Pentalitomastix sp. recovered from 40-day-old A. kuehniella larva. Imaginal discs are apparent on the anterior third of the body.

tacea for 24 h and then to Pentalitomastix sp. for another 24 h each yielded one or more live precocious larvae and one wounded and dead P. flavitestacea larva. In 70% of these cases, the latter parasite was partially or entirely encapsulated (Fig. 3), indicating that an immune response, a common defense reaction in insects6, had been elicited in the host larva. For the reversed sequence of parasite exposure, 64% of 50 multiply parasitized hosts yielded wounded, dead and encapsulated P. flavitestacea larvae.

In contrast, no wounded or encapsulated parasite larvae were found in 250 A. kuehniella larvae exposed as eggs to P. flavitestacea alone. In each of 14 observed cases of superparasitism, encounters among P. flavitestacea larvae occurred within 1 or 2 days of hatching of the larvae, at which time the host larva was still within its chorion. These encounters were fatal to all but one parasite larva and the losers were dismembered, not merely wounded. When 65 multiply parasitized host larvae were dissected before formation of the precocious larvae, the P. flavitestacea larvae were found alive. These observations, together with the extreme motility and heavily sclerotized buccal structures of the precocious larvae, indicate that they attack and kill P. flavitestacea larvae. This could explain why in laboratory tests, Ectomyelois ceratoniae (Zeller) multiply parasitized by P. flavitestacea Fischer and Pentalitomastix plethoricus Caltagirone reportedly gave rise to the latter parasite alone7

A. kuehniella larvae multiply parasitized by Pentalitomastix sp. and Trathala sp. yielded wounded, dead and encapsulated larvae of the latter and one or more precocious larvae of the former. Despite the similarity in appearance and motility of these larvae, the precocious larvae 'won' in all 29 cases (Fig. 4). In each of six cases of superparasitism by Trathala sp., all parasite larvae but one were found wounded, dead and encapsulated. In nine hosts superparasitized by Trathala sp. and additionally parasitized by Pentalitomastix sp., no Trathala sp. larvae survived when precocious larvae were present.

The presence of castes and intergenerational cooperation characterizes eusocial insects8. Although the polyembryonic

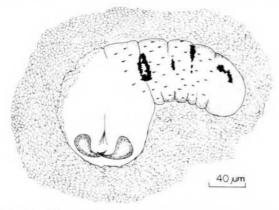


Fig. 3 Partially opened capsule containing dead, wounded, firstinstar Phanerotoma flavitestacea larva. Wounds appear as melanized lesions on the integument. The capsule consists of layers of cells that seem to have been laid down concentrically around the dead larva.

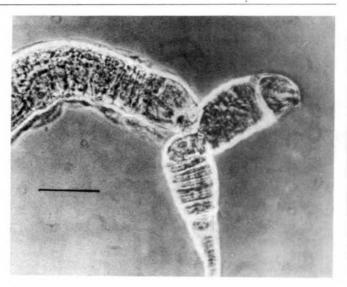


Fig. 4 Precocious larva of Pentalitomastix sp. (left) attacking larva of Trathala sp. Specimen was recovered from 49-day-old A. kuehniella larva exposed as an egg to Pentalitomastix sp. and as a 21-day-old larva to *Trathala* sp. Polaroid photograph of fresh specimen in Ringer's saline viewed through Tiyoda phase-contrast microscope. Scale bar, 0.2 mm.

Encyrtidae are not eusocial, the developmental pattern of known species involves the obligatory coexistence of a large number of individuals in a host vulnerable to attack by other internal parasites. Furthermore, any brood of parasites arising by polyembryony exists as a physically defenseless polygerm for an extended period during its own life and that of the host. Therefore, I suggest that the precocious larva serves as a defender morph and provides a means of increasing the competitive advantage and probability of survival of polyembryonic encyr-

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## The path of axons in *Drosophila* wings in relation to compartment boundaries

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We have tested the hypothesis that the paths taken by sensory axons in the peripheral nervous system of Drosophila are influenced by the boundaries between developmental compartments<sup>1</sup>. It has been proposed that differences in cell affinity keep the epidermal cells of adjacent compartments from intermingling2. Such differences in cell affinity might well also prevent the axons of sensory neurones, which are derived from the same populations as epidermal cells and which use them as growth substrates, from crossing compartment boundaries. However, as we report here, we have found that sensory axons in the wing of Drosophila do cross the boundary between the anterior and posterior compartments of the wing.

The thoracic segments of Drosophila melanogaster are each divided at least into anterior and posterior (A and P) compart-

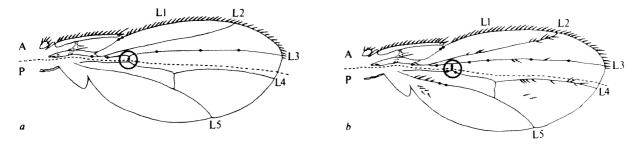


Fig. 1 Morphology of the wing of *D. melanogaster. a*, Wild type, with receptors on specific veins; *b, Hairy wing (Hw)*, with supernumerary receptors in many locations. L1-L5, longitudinal veins 1 to 5; •, large campaniform sensilla; :::, small campaniform sensilla on the radial vein; ---, A-P compartment boundary; heavy circle surrounds the area of the anterior cross vein (ACV), shown enlarged in Fig. 2. The compartment boundary in *Hw* animals was determined from the behaviour of mitotic recombination clones marked with *y* and *mwh* in the progeny of two crosses: y/y;  $mwh/mwh \times y Hw/Y$ ;  $Dp sc^{14}M(3)i^{55}/mwh^+$  and y Hw/FM6;  $mwh/mwh \times y Hw/Y$ ;  $Dp sc^{14}M(3)i^{55}/mwh^+$ . Egg collections were made over a 24-h period, and larvae aged 24-48 h were irradiated with a dose of 1,000 R (for further details, see ref. 6). Clones in flies homo-, hetero- and hemizygous for Hw were analysed and all behaved like similar clones in wild-type animals<sup>5</sup>. (For explanation of symbols and description of mutants, see ref. 16.)

ments. The presence of compartments is recognized because genetically marked clones of cells induced after a certain time in development never cross particular lines on the body surface. On the wing, the A-P boundary runs a few cells anterior to the fourth longitudinal vein<sup>1</sup> (L4; Fig. 1a).

There is evidence that compartments do influence the paths followed and the connections formed by sensory axons in the central nervous system. The particular route taken by an axon apparently depends not only on the type of receptor from which it originates, but also on the developmental compartment in which its cell body is located<sup>3</sup>. Furthermore, the reflex responses elicited by stimulation of similar receptors seem to depend on the compartment in which the sensory cell bodies lie<sup>4</sup>. Here we have investigated whether the organization of peripheral axonal pathways is also influenced by compartments.

All the known receptor cells in the wings of wild-type Drosophila are located in the anterior compartment (Fig. 1a)<sup>5,6</sup>. To examine the hypothesis that the A-P compartment boundary of the wing forms a barrier to axon growth, one needs receptors in the posterior compartment whose axons could potentially join the anterior nerve bundles. Flies carrying  $Hairy\ wing\ (Hw)$ , a dominant, sex-linked mutation, provide suitable material for study. This mutation causes both sensory bristles and campaniform sensilla (disk-shaped cuticular strain detectors) to form in many regions of the cuticle normally devoid of receptors, including the posterior compartment of the wing. Supernumerary receptors are found on veins L2, L3, L4, L5, the anterior cross vein and occasionally on the radial vein, as well as in the inter-vein areas (Fig. 1b).

The anterior cross vein (ACV) connects vein L4 (posterior) with vein L3 (anterior), and thus forms a potential conduit for axon growth between the two compartments. In wild-type flies this vein carries a single sensillum, located in the anterior compartment (Fig. 2a). Its cell body usually lies at or near the L3-ACV junction, and its dendrite travels through the ACV to attach to the dome of the sensillum. In Hw flies, one or more supernumerary sensilla may be present on the ACV, and extra sensilla may also occur on L4 near the ACV (Fig. 2b-d). In all such cases we have analysed, the axons pass through the ACV to join the nerve travelling through L3. If the cell body lies in L4 distal to the ACV (Fig. 2c), its axon makes a right angle turn to enter the ACV. If the cell body is located proximal to the ACV (Fig. 2d) its axon, in addition, first travels distally, the opposite of the usual direction. The preference of supernumerary axons in this region for following the ACV and L3 is not due to the inability of L4 to support axon growth. This is evident because the axons of distal receptors travel successfully in L4 before turning into the ACV, and receptors nearer to the proximal end of the vein commonly send their axons as a bundle through it towards the base of the wing.

Even though the nerve bundles formed by the more proximal receptors often travel towards the base of the wing, both single axons and large nerve bundles can also grow at right angles to the veins, cross the compartment boundary and join the major nerves in L3 or the radial vein (Fig. 3). The supernumerary receptors in these more proximal regions of the posterior compartment include many bristles as well as campaniform sensilla. Thus, the axons of both receptor classes readily cross the A-P boundary, and crossing is not confined to any particular location on the wing. The ACV provides an open channel, but well defined veins are not required for axons to cross into the anterior compartment.

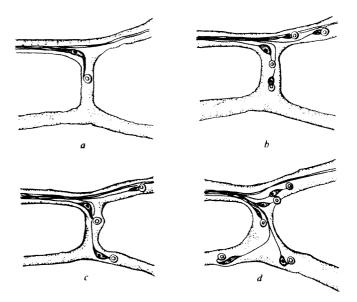
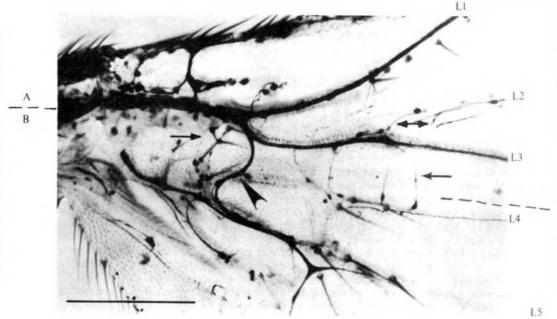


Fig. 2 Course of axons in the region of the anterior cross vein (ACV). a, Wild-type fly with one campaniform sensillum, its cell body at the ACV-L3 junction and its axon travelling through L3. Proximal is to the left in each drawing, distal to the right. b-d, Examples of homozygous Hw animals. Campaniform sensilla and their cell bodies are found in the posterior compartment, and their axons turn into the ACV, cross the compartment boundary and join the nerve bundle in L3. Hemizygous males and heterozygous females, which have fewer supernumerary receptors, show similar patterns. Drawn from preparations stained with methylene blue. Stain (Methylene Blue Ehrlich, Chroma,  $0.05 \text{ g ml}^{-1}$ ) was injected into the thorax of adult flies and the wings were removed after 0.5-4 h and mounted in a drop of mineral oil. Contrast could often be enhanced by using differential interference optics. Over 100 wild-type and mutant flies with adequate staining were analysed.

Fig. 3 Course of axons in the proximal wing of a homozygous Hw fly. A massive nerve bundle from the posterior compartment (◄) joins the normal anterior nerves at the confluence of veins L1 and L3. Numerous single axons cross from L4 to L3 (←), from scattered posterior receptors to the radial vein (→), and from L2 to L3 (↔). The location of the compartment boundary is indicated by the dashed lines in the margin (compare with Fig. 1). Methylene blue staining as in Fig. 2. Scale bar, 200 µm.



We have used Hairy wing flies to probe the potential barrier to axon growth at the A-P compartment boundary. However, it is conceivable that the Hw mutation, in addition to producing supernumerary receptors, might shift or eliminate the very A-P compartment boundary whose properties we want to analyse. If this were the case, sensilla which we believe to be in the posterior compartment on the basis of their location would actually be anterior in character and their axons would not be crossing a compartment boundary. To test this possibility, we induced large clones of cells marked with the mutation multiple wing hairs (mwh) in Hw flies (see Fig. 1 legend). These clones were confined to the same areas, and defined the compartment boundary in the same location, as in wild-type flies in all the genotypes we used for axon tracing: Hw/Hw females, Hw/+ females and Hw/Y males. Thus, there is no indication that Hwaffects the compartment boundary. Furthermore, if the existence of the boundary is the outcome of affinity differences between anterior and posterior cells, these differences must also be present in Hw flies and must be irrelevant to the growth of adult axons.

Our clones were marked with the mutation yellow (y) in addition to mwh, so that the sensory bristles could also be scored. We never found y bristles in wild-type tissue or wildtype  $(y^+)$  bristles in a y clone. These observations argue strongly against the possibility that anterior bristle precursor cells have migrated into the posterior compartment.

Thus, adult axons can freely cross the A-P compartment boundary. On their way to the central nervous system they also cross at least the proximo-distal boundary in the wing. On the other hand, Lawrence<sup>8</sup> reported that sensory axons do not cross segment borders in the abdomen of the milkweed bug Oncopeltus. Furthermore, he showed that clones in the epidermis are restricted early in development to an individual segment. Thus, the segment border is a compartment border on this criterion. However, the present demonstration that the A-P compartment boundary does not constitute a barrier to the growth of axons indicates that in this respect segment borders have properties which not all other compartment boundaries share.

The developmental history of axon growth in the wing is incompletely known, but the course of axons in the adult is presumably a consequence of a pattern laid down earlier. Waddington9 reported the presence of 'nerve bundles' in veins L1 and L3 of the metamorphosing wing before the differentiation of the adult sensory cells. Preliminary studies using the electron microscope 10 indicate that these structures are much

more complex than he realized, but the time of their appearance and their localization are reminiscent of the pioneer axons which have more recently been described in other insect appendages<sup>11-13</sup>. Experimental evidence for the causal role of such axons in the organization and placement of peripheral nerves is now available14. Whatever guiding factors are present in the wing, however, be they 'pupal nerves', pioneer axons, or specific non-neural substrates, we expect them to be localized primarily in veins L1 and L3. Not only are these the veins in which adult nerves are normally present, but axons from ectopic receptors often leave a particular vein at apparently arbitrary points to join the fibres travelling in L1 and L3. Figure 3 shows one example of the many we have seen of axons crossing from both L2 (an anterior vein) and L4 (a posterior vein) to join the nerve in L3.

We consider it a reasonable working hypothesis that the filopodia of growing adult axons explore the nearby territory and if a suitable guiding factor is available they follow it preferentially and with the correct polarity. If the normal guiding factor is not available, other features of the environment may influence the growth of the axon but correct polarity may not be achieved (unpublished observations). In this process, compartments and the boundaries between them appear not to be important.

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# Evidence for expression of the paternal genome in the two-cell mouse embryo

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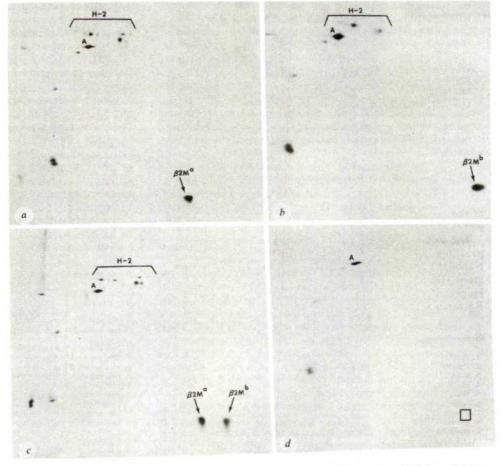
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Although there is strong evidence that the paternal genome is activated at the four- to eight-cell stage of mouse embryogenesis1-12, studies to date have been restricted because of their dependence on the manifestations of gene products, such as enzyme activity and cell-surface expression, rather than on direct assay of their synthesis.  $\beta_2$ -microglobulin ( $\beta_2$ M), a 12,000-molecular weight  $(M_r)$  peptide associated with several of the gene products encoded by the H-2-Tla complex in the mouse  $^{13-17}$ , is synthesized by early cleavage stage embryos (J.A.S., T.M. and C.J.E., unpublished). The existence of an electrophoretic variant of \( \beta\_2 M \) in inbred strains has recently been described18-20, and tryptic peptide mapping19 and amino acid sequencing data21 indicate that the primary sequences of these variants differ by a single amino acid, thus establishing the genetic basis for this variation. Mice heterozygous at the  $\beta_2$ m locus synthesize both forms,  $\beta_2 M^a$  and  $\beta_2 M^b$  (refs 18, 20). Using direct immunoprecipitation and two-dimensional gel electrophoresis, we have used this variation at the  $\beta_2$ m locus to distinguish between maternal and paternal \( \beta\_2 M \) and have now established that the synthesis of paternally derived  $\beta_2 M$  is first detectable at the two-cell stage. This is the earliest stage at which expression of the mammalian embryonic genome has been established22.

Autoradiograms Fig. immunoprecipitated polypeptides from <sup>35</sup>S-methionine-labelled lymphocytes from the splenic parental inbred strains. a, SWR/J lymphocytes, anti- $\beta_2 M$ ; C57BL/6, anti-\(\beta\_2\text{M}\); c, SWR/J anti-BaM+ lymphocytes, C57BL/6 lymphocytes, anti-β<sub>2</sub>M; d, SWR/J lymphocytes, preimmune serum. Radiolabelling of lymphocytes and preparation of gels were done as previously described<sup>23</sup>. Briefly, lymphocytes were isolated and labelled for 6 h (3×10<sup>7</sup> cells per ml per 250μCi 35S-methionine) in Dulbecco's modified Eagle's medium lacking methionine. Aliquots of cell extracts prepared in 0.5% NP40 were reacted with antiserum for 15 min at 4 °C after which a suspension of S. aureus protein A (10% wt/vol) (Enzyme Center) was added and the mixture was incubated for an additional 10 min at 4°C. Protein A-bound immune complexes were released by the addition of O'Farrell's lysis buffer<sup>24</sup>. The immunoprecipitates were analysed on two-dimensional gels. Non-equilibrium pH gradient gels having an approximate pH range of 4.5-7.9 were used as the first dimension<sup>25</sup>. Electrophoresis was carried out for 4 h at 400 V. Separation in the second dimension was done using a 4.5% polySWR/J superovulated female mice carrying the  $\beta_2 m^a$  allele were mated to C57BL/6 males carrying the  $\beta_2 m^b$  allele, and standard procedures were used to obtain fertilized eggs and preimplantation mouse embryos. Embryos from the reciprocal mating (C57BL/69×SWR/Jd) were also collected. The embryos were radiolabelled with <sup>35</sup>S-methionine, protein extracts were prepared and reacted with a rabbit anti-mouse  $\beta_2$ M serum and the antigen-antibody complexes were precipitated with protein A derived from Staphylococcus aureus, Cowan I strain. After release of the complexes from the immunoabsorbent, the immunoprecipitates were analysed by two-dimensional polyacrylamide gel electrophoresis and autoradiography.

Three criteria are used to verify the identity of the  $\beta_2 M^a$  and  $\beta_2 M^b$  peptides on the two-dimensional gels: (1) The peptides are not precipitated by preimmune rabbit serum. (2) Each peptide has a  $M_r$  of 12,000 and an isoelectric point of 6.7. The apparent  $M_r$  of  $\beta_2 M^a$  is slightly higher than that of  $\beta_2 M^b$ , while the apparent isoelectric point of  $\beta_2 M^b$  is slightly higher than that of  $\beta_2 M^a$  (Fig. 1). (3) Each peptide co-migrates with a purified preparation of either  $\beta_2 M^a$  or  $\beta_2 M^b$ .

Using this procedure, we tested fertilized eggs and two-, four- and eight-cell embryos heterozygous at the  $\beta_2 m$  locus and determined that the synthesis of paternally derived  $\beta_2 M$  is detectable as early as the two-cell stage (Fig. 2c). However, although  $\beta_2 M$  synthesis is readily detectable in unfertilized eggs (unpublished), we were unable to detect the synthesis of either maternally or paternally derived  $\beta_2 M$  in fertilized eggs (Fig. 2a, b). This finding, coupled with the observation that maternally and paternally derived  $\beta_2 M$  are apparently synthesized in approximately equal amounts in two-, four- and eight-cell embryos (Fig. 2c, d), suggests that maternally derived  $\beta_2 M$  messenger RNA is either degraded or otherwise inactivated at the time of fertilization and that the observed synthesis of  $\beta_2 M$ 



acrylamide stacking gel and a 14% polyacrylamide separation gel. The square in d indicates the site to which  $\beta_2 M$  would migrate on the gel. 'A' indicates actin, which has a  $M_\tau$  of 44,000. The basic side of each gel is on the right.

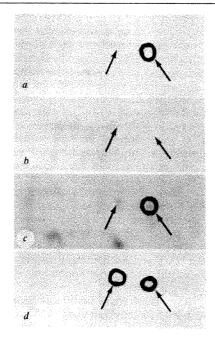


Fig. 2 35S-methionine-labelled peptides immunoprecipitated with rabbit anti-mouse  $\beta_2 M$  serum from: a, fertilized eggs, SWR/J $\circ$  C57BL/6 $\circ$ ; b, fertilized eggs, C57BL/6 $\circ$  SWR/J $\circ$ ; c, two-cell embryos, C57BL/6 $\circ$  SWR/J $\circ$ ; d, four-cell embryos, C57BL/6 $^{\circ}$  ×SWR/J $^{\circ}$ . The panels represent comparable regions from four two-dimensional gel autoradiograms. For each gel, 200-400 fertilized mouse eggs or preimplantation mouse embryos were obtained from superovulated females. Embryos were transferred to 30 µl of modified Whitten's medium<sup>26,27</sup> containing <sup>35</sup>S-methionine (specific activity 800-1,200 Ci mmol<sup>-1</sup>) (Amersham/Searle) at a concentration of 2 mCi/ml<sup>-1</sup> and cultured under paraffin oil at 37 °C in a 5% CO<sub>2</sub> atmosphere for 4 h. At the end of the incubation period, embryos were solubilized in 100 µl of 0.5% NP40 (Particle Data Inc.) in Tris-buffered saline (150 mM NaCl, 50 mM Tris, 0.02% NaN<sub>3</sub>, pH 7.0). Each extract was centrifuged at 10,000g for 5 min in a Beckman microfuge B to remove unlysed nuclei, and the supernatants were stored at -80 °C. Immunoprecipitates were prepared as described in Fig. 1 legend. In a and c, purified mouse  $\beta_2 M^b$  (2.75 µg) was added to each immunoprecipitate sample before electrophoresis, while in d, both purified  $\beta_2 M^a$  $(2.75 \mu g)$  and  $\beta_2 M^b$   $(2.75 \mu g)$  were added. Proteins were fixed and stained with 50% trichloroacetic acid-0.1% Coomassie blue, the gel was dried, and the stained  $\beta_2 M$  spot(s) was circled with radioactive ink. The arrows in a and b indicate the absence of  $\beta_2 M^a$ and  $\beta_2 M^0$ , while the arrows in c and d indicate their presence. Each gel was exposed to X-ray film for  $\sim 2 \times 10^6$  count-days (c.p.m. loaded on to gel × no. of days exposed).

at the two-cell stage is in fact the result of activation of the embryonic genome.

The rabbit anti-mouse  $\beta_2 M$  serum and purified  $\beta_2 M$  preparations were provided by Dr Ettore Appella from the National Cancer Institute. This work was supported by NIH grant HD-03132. C.J.E. was an investigator of the Howard Hughes Medical Institute. T.M. is a recipient of a NIH National Research Service Award.

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## Regulation of epithelial tight junction permeability by cyclic AMP

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In 'leaky' epithelia, ions move through both a transcellular and a paracellular (serial alignment of tight junction and intercellular space) path. The efficiency of transepithelial transport could therefore be regulated if the cell was able to alter reversibly the permeability of tight junctions. (These are specialized regions of the apical cell membranes common to all epithelia.) We now report that such a mechanism indeed exists in the Necturus gallbladder. It is effected by cyclic AMP, which is already known to mediate surface membrane phenomena in a variety of cell systems through its interaction with the cytoskeletal system<sup>1,2</sup>, In gallbladders mounted and perfused with electrolyte solutions in an Ussing-type chamber, exposure of the mucosal surface to cyclic AMP analogues increased transepithelial electrical resistance, potential difference and short-circuit current and decreased NaCl dilution potentials in a rapid and reversible manner. We also observed rapid depolarization of cell membrane electrical potentials and a slow decline in intracellular K+ activity. Freeze-fracture electron microscopy of tissues fixed with glutaraldehyde during the peak electrical response showed a reorientation of intramembranous junctional fibrils, suggesting that cyclic AMP reduces the ionic permeability of the paracellular pathway in this epithelium by altering the structure of tight junctions.

After decapitating the animals, gallbladders were removed, washed and mounted mucosal side up between two halves of a Plexiglass chamber. The tissue was loosely stretched over a 0.13-cm<sup>2</sup> chamber opening and sealed with vacuum grease between chamber halves. Tissues were bathed on both sides by a continuous flow of amphibian Ringer's solution. Transepithelial resistance  $(R_t)$ , spontaneous electrical potential difference  $(\psi_{ms})$ and short-circuit current (Isc) were measured with the fourelectrode technique described in Fig. 1 legend. Mucosal cell membrane electrical potentials ( $\psi_{mc}$ ) were measured with a high-impedance electrometer by advancing a microelectrode  $(1 \text{ M KCl}, > 10 \text{ M}\Omega)$  tip resistance) into the cells from the mucosal bath. NaCl dilution potentials were measured as the change in steady-state  $\psi_{ms}$  observed when one-half of the NaCl in the mucosal bath was replaced isosmotically with sucrose. No correction was made for liquid junction potentials because control, experimental and recovery measurements were made with the same solutions for the same gallbladder.

The gallbladders were also studied morphologically. They were fixed in situ by simultaneously applying a phosphatebuffered 2% glutaraldehyde solution to the mucosal and serosal surfaces. Freeze-fracture was performed at -100 °C in pre-

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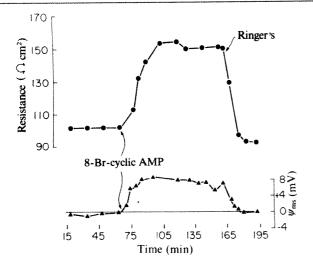


Fig. 1 Time course of a typical experiment showing a reversible increase in transepithelial electrical potential difference ( $\psi_{ms}$ ,  $\triangle$ ) and resistance ( $\bigcirc$ ) on addition of  $10^{-3}$  M 8-Br-cyclic AMP to mucosal bath of gallbladder mounted in an Ussing-type chamber. Composition of bathing solution (mM): NaCl, 87; KCl, 4.5; CaCl<sub>2</sub>, 1.8; MgCl<sub>2</sub>, 1.0; KH<sub>2</sub>PO<sub>4</sub> 0.7; NaHCO<sub>3</sub>, 7.5; HEPES buffer, 5.0; pH 7.4, when equilibrated with 99% O<sub>2</sub>, 1% CO<sub>2</sub> at 25 °C. Transepithelial electrical potential difference ( $\psi_{ms}$ , measured with respect to the mucosal bath, bottom trace), transepithelial resistance ( $R_t$ , top trace) and short-circuit current ( $I_{sc}$ , not shown in figure) were measured by an automatic voltage-clamp electrometer in which solution resistance was automatically subtracted.  $\psi_{ms}$  was measured by calomal electrodes using 1 M NaCl or 3 M KCl-filled agar bridges;  $R_t$  was measured by passing 100- $\mu$ s bipolar current pulses (75  $\mu$ A cm<sup>-2</sup>) through the tissue via Ag/AgCl electrodes; and  $I_{sc}$  was measured by passing a current sufficient to reduce  $\psi_{ms}$  to zero.

viously fixed tissue impregnated with 20% glycerol (Denton DFE-3 apparatus) and examined with a Siemens 1A electron microscope.

Control electrophysiological parameters are summarized in Table 1 and are in general agreement with those measured by other investigators<sup>3-5</sup> who have also noted a low  $R_t$  in this epithelium. Figure 1 shows a typical experiment. After the baseline  $\psi_{\rm ms}$  and  $R_t$  stabilized, the mucosal bathing solution was replaced by an identical Ringer's solution containing  $10^{-3}$  M 8-bromo-cyclic AMP (8-Br-cyclic AMP).  $R_t$  and  $\psi_{\rm ms}$  began to increase within 5-10 min and peaked by 30-75 min. Reversibility was indicated by a return of electrical parameters to baseline when the 8-Br-cyclic AMP mucosal bathing solution was replaced by the standard Ringer's solution. Note that, within 5-10 min, 8-Br-cyclic AMP depolarized  $\psi_{\rm mc}$  and  $\psi_{\rm cs}$  (the basolateral membrane potential), while  $R_{\rm m}/R_{\rm s}$  decreased and  $I_{\rm sc}$  increased (Table 1). The decline in K<sup>+</sup> activity  $(a_{\rm K}^+)$  was

gradual during exposure to 8-Br-cyclic AMP. When the mucosal bathing solution was again replaced with the control electrolyte solution (recovery), all parameters, except  $a_{\rm K}$ , which rose to a value above the control value, returned within 15 min to near control values. In four separate tissues, mucosa positive dilution potentials (with respect to serosa) were reduced from  $15.3 \pm 0.4 \, {\rm mV}$  (control) to  $12.0 \pm 0.9 \, {\rm mV}$  (8-Br-cyclic AMP) and returned to  $13.9 \pm 0.8 \, {\rm mV}$  (recovery), P < 0.01.

In dose-response experiments, we determined the fractional peak change in transepithelial resistance  $(\Delta R_t)$  to various mucosal concentrations of 8-Br-cyclic AMP.  $\Delta R_t$  increased linearly with increasing mucosal concentrations. At 10<sup>-5</sup> M (n=3) there was no change in  $R_t$  and at  $10^{-2}$  M (n=3)  $R_t$ increased to 1.5 times the control value. A comparable  $\Delta R_t$  was observed when 8-Br-cyclic AMP was dissolved in the serosal bath, except that response and recovery were delayed. On a molar basis, the cyclic AMP analogue 8-p-chlorophenylthiocyclic AMP produced a greater response than 8-Br-cyclic AMP; dibutyryl cyclic AMP produced a lower response. 10<sup>-3</sup> M isobutyl-1-methylxanthine, a phosphodiesterase produced a 12% increment in R. However, a 25% increment was observed when the mucosal tissue surface was pretreated with 10<sup>-5</sup> M 8-p-chlorophenylthio-cyclic AMP, a concentration which by itself did not increase resistance. 8-p-chlorophenylthio-cyclic GMP  $(10^{-5}-10^{-4} \text{ M})$  reduced  $R_t$ , but the dose-response relationship was not linear and  $\psi_{\rm ms}$  did not change.

In thin-section electron micrographs, gallbladders treated with  $10^{-3}$  M 8-Br-cyclic AMP demonstrated thickening and aggregation of microfilaments in submembranous regions, particularly those adjacent to tight junctions. Epithelia fixed with glutaraldehyde within 5 min of exposure to 8-Br-cyclic AMP ( $10^{-3}$  M) and freeze-fractured showed basal strands in focal regions extending well below the main fibrillar meshwork. By 30 min (peak effect) this finding was more pronounced with occasional isolated strands appearing below the meshwork, resulting in an increase in number of strands and a longitudinal lengthening of the junction (Fig. 2). These morphological changes are expressed semiquantitatively by morphometric analysis (Table 2). Intercellular space morphology did not show appreciable change.

If an external current is applied across *Necturus* gallbladder, >90% of the current flows paracellularly<sup>3,6</sup> through the tight junction and intercellular space in series. Therefore,  $R_t$  is dominated by the ionic conductance of the paracellular pathway. Na<sup>+</sup> absorption in this tissue is characterized by an electroneutral NaCl-coupled mucosal influx<sup>4</sup> and the leaky paracellular shunt, both of which are responsible for the observed near-zero  $I_{sc}$  and  $\psi_{ms}$  during control conditions. The increase in  $R_t$ ,  $I_{sc}$  and  $\psi_{ms}$  observed in 8-Br-cyclic AMP-treated tissues is consistent with reduced paracellular shunting, probably due to a reduced ionic permeability of the tight junction. A chemical or physicochemical alteration of fixed charge within the transjunctional path might decrease Na<sup>+</sup> or increase Cl<sup>-</sup> conductance, causing the observed reduction in dilution potentials. On

	7	Table 1 Electron	physiological parame	ters of gallbladder er	oithelium		
	$\psi_{ms}(mV)$	$R_{r}(\Omega \text{cm}^{2})$	$I_{\rm sc}(\mu \text{A cm}^{-2})$	$\psi_{\rm mc}({ m mV})$	$R_{ m m}/R_{ m s}$	$\psi_{cs}(mV)$	$a_{\mathbf{K}^+}(\mathbf{m}\mathbf{M})$
Control 8-Br-cyclic AMP Recovery	$-0.7 \pm 0.3$ $6.9 \pm 0.9$ $-0.1 \pm 0.3$	$86 \pm 11$ $127 \pm 11$ $82 \pm 6$	$-5.4 \pm 4.3$ $64.7 \pm 16.8$ $-1.5 \pm 4.0$	$-50 \pm 2 (30)$ $-38 \pm 0 (24)$ $-50 \pm 2 (21)$	$\begin{array}{c} 2.56 \pm 0.36 \\ < 0.10 \\ 1.24 \pm 0.20 \end{array}$	49±2 45±1 51±2	81±5 68±2 90±7

Mean  $\pm$  s.e.m. from five experiments. Numbers in parentheses signify the number of successful microelectrode impalements.  $\psi_{cs}$  is basolateral membrane potential calculated from  $\psi_{ms} = \psi_{mc} + \psi_{cs}$ . The ratio of mucosal to basolateral membrane resistance  $(R_m/R_s)$ , the voltage divider ratio) was calculated from  $\Delta\psi_{mc}$  and  $\Delta\psi_{ms}$ , corrected for solution resistance, measured during application of 100- $\mu s$  current pulses <sup>6.10</sup>. Intracellular K<sup>+</sup> activity,  $a_{K}^+$ , was measured by K<sup>+</sup>-selective microelectrodes fabricated with Corning 47715 K<sup>+</sup> liquid ion exchanger by methods described <sup>11</sup>. Calibration plots in KCl standard solutions yielded an average slope of  $56.2 \pm 2.3$  mV per decade K<sup>+</sup> for 18 electrodes. Average values after  $10^{-3}$  M 8-Br-cyclic AMP were recorded at peak resistance, 30-75 min after its addition to the mucosal bath. Values for the recovery period were recorded within 15 min after removal of 8-Br-cyclic AMP. Peak values are significantly different (at least P < 0.05 by paired t-test) from control and recovery, except  $\psi_{cs}$  with respect to control.

Table 2 Morphometrical analysis of tight junctions

Condition	n	No. of horizontal strands	Linear density of horizontal strands (strands per µm)	Tight junction depth (μm)
Control	102	$8.0 \pm 0.2$	$16.1 \pm 0.5$	$0.56 \pm 0.02$
8-Br-Cyclic AMP (5 min)	43	$8.0\pm0.3$	$14.9\pm0.6$	$0.56\pm0.02$
8-Br-cyclic AMP (peak response)	65	$10.4 \pm 0.5$	$14.0 \pm 0.6$	$0.79 \pm 0.04$
Recovery	60	$8.9\pm0.3$	$14.8\pm0.5$	$0.62 \pm 0.02$

Values are mean ± s.e.m.; n is the number of grid lines drawn perpendicular to the most luminal junctional strand at 0.5-cm intervals on electron micrographs (×60,000). The average frequency of intersection of strands with grid lines determines the number of horizontal strands. Tight junction depth is the mean distance from the most luminal to most abluminal strand. 10<sup>-3</sup> M 8-Br-cyclic AMP was used in all experiments. All changes measured at peak response were significantly different from control and recovery (at least P<0.05 by unpaired t-test), except for the change in linear density with respect to recovery. Cyclic AMP induces a reversible longitudinal expansion of the fibrillar

the other hand, reduction of dilution potentials by cyclic AMP may simply reflect a change in dimensions of a normally cationselective channel.

Cyclic AMP has additional effects on cell membrane transport in this epithelium. 8-Br-cyclic AMP may increase Na+ conductance of the mucosal membrane, as demonstrated by the depolarization of  $\psi_{mc}$  and decreased ratio of mucosal to basolateral membrane resistance. Assuming no change in Rs, we calculate that mucosal membrane conductance increased 25fold. This is consistent with our observation that  $\Delta \psi_{\mathrm{mc}}$  virtually disappeared after exposure of the mucosal surface to 8-Br-cyclic AMP. Depolarization of  $\psi_{\rm mc}$  (and  $\psi_{\rm cs}$ ) and an increase in  $\psi_{\rm ms}$ with increased mucosa to serosa Na flux (and Isc) have been observed in Necturus gallbladder exposed to the antibiotic



Fig. 2 Freeze-fracture of gallbladder epithelium fixed at peak response (10<sup>-3</sup> M 8-Br-cyclic AMP). Note the increased number of grooves below the meshwork. Microvilli (MV) are labelled (×45,000). In control gallbladders fixed while bathed in amphibian Ringer's solution, strands form a regular meshwork. Occasionally an abluminal strand will extend down from this meshwork (see ref. 7).

amphotericin B, a drug thought to increase mucosal membrane conductance to cations4.5. However, increased mucosal membrane conductance cannot account for the observed decrease in NaCl dilution potentials or increased R, which require an independent effect on tight junction conductance. An electrical circuit model of Necturus gallbladder epithelium predicts that an increase in the shunt pathway resistance alone will depolarize  $\psi_{mc}^{3}$ . However, this model also predicts a hyperpolarization of the  $\psi_{cs}$  in these conditions rather than the 4-mV depolarization observed here. The gradual decrease in  $a_{K^+}$  after mucosal exposure to cyclic AMP is consistent with a change in cell membrane permeability to K+ or may result from the observed cell membrane depolarization. Thus, intracellular electrolyte shifts and/or changes in cell membrane permeability may account for our failure to observe the predicted hyperpolarization of  $\psi_{cs}$ .

The exact mechanism of cyclic AMP modulation of tight junctional permeability is not defined. Plant cytokinins behave electrophysiologically like a phosphodiesterase inhibitor while altering microfilament morphology7. As cyclic AMP reacts with the cytoskeleton in other cell systems, it may have a specific role in the orientation of tight junctional intramembranous strands, thereby regulating paracellular ion flow. Uncertainties as to the nature of the transjunctional ionic pathway restrict speculation on structure-function correlations. However, in a simple conductor, resistance is directly proportional to length and in these experiments resistance increased ~50% while junctional depth increased ~40%. Cyclic AMP may not affect all leaky epithelia in the same way; indeed, in mammalian kidney proximal tubule it increases transepithelial 14C-sucrose flux8, while in rabbit ileum it increases transepithelial electrical resistance9

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## Increase in faecal nitrosamines in Japanese individuals given a Western diet

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Colon cancer is prevalent in North America and Western Europe, but has a low incidence in Japan<sup>1,2</sup>. Epidemiology suggests that a high intake of animal fat<sup>3,4</sup> and protein<sup>5,6</sup>, especially beef<sup>7,8</sup>, is associated with colon carcinogenesis. Numerous hypotheses have been proposed to account for the relationship between these diets<sup>9-13</sup>, but experimental support for them is unconvincing. The specific carcinogen involved in cancer of the large bowel remains to be identified experimentally. Bruce and co-workers14 showed that several volatile nitrosamines were present in normal human faeces and suggested that these were produced endogenously in the lower intestine but they subsequently negated this hypothesis 15. We demonstrate here that the levels of volatile nitrosamines in human faeces are markedly increased in Japanese individuals given a Westernstyle diet, but decreased by a typical Japanese diet.

We studied six healthy Japanese male volunteers from our laboratory (aged 27-39 yr) who were not receiving medical treatment during this study. Three of the subjects were given, in order: (1) a typical Japanese diet for 4 days; (2) a mixed Japanese diet for 3 days; (3) a balanced Western-style diet for 8 days and then a typical Japanese diet for 6 days. Four of the subjects were given, in order, a long-term mixed Japanese diet followed by a high-fat, high-meat diet for 4 days. These diets are shown in Table 1. The typical Japanese diet consists of boiled rice and side dishes of fish and vegetables. This diet provided ~2.0 kcal, 15% protein (mostly cereal protein), 20% fat, 65% carbohydrate and 75 mg vitamin C. In the case of the balanced Western-style diet, the staple food is meat, with side dishes of bread and vegetables. For breakfast, orange juice was replaced by vegetables to provide nitrate intake. This diet provided

	Table 1 Composition of diets					
	Food item	Amount				
Typical Japa		1				
Breakfast	Rice, boiled	1 cup				
	Soup, containing bean paste (miso) and bean curd	1 cup				
	(tofu), vegetable or seaweed Egg, raw or cooked	1				
	Seaweed (dried laver (nori)) and/or vegetable	5 pieces				
	(Chinese cabbage, spinach, etc.), boiled	50 g				
	Pickled vegetable	Little				
	Japanese green tea	1/2 cup				
Lunch	Rice, boiled, or noodles	2 cups 1 cup				
	Soup (similar to breakfast) Fish, broiled	70 g				
	Vegetable (same as breakfast)	50 g				
	Pickled vegetable	Little				
	Fruit (Japanese mandarin)					
	Japanese green tea	1 cup				
Dinner	Rice, boiled	2 cups 1 cup				
	Soup (similar to breakfast)	70 g				
	Fish, broiled, boiled, fried or raw Cooked vegetables (4-5 kinds of raddish, carrot,	1 cup				
	burdock, onion, potato and mushroom (shiitake),					
	added fish paste or chicken)					
	Pickled vegetable					
	Fruit (apple or Japanese mandarin)	1 /2				
	Japanese green tea	1/2 cup				
Balanced W	/estern-style diet					
Breakfast	Egg and bacon or ham, fried	150 g				
	Bread and butter	1 slice 100 g				
	Vegetables (1-2 kinds of lettuce, celery and spinach),	100 g				
	salad or fried Whole milk	1 cup				
	Coffee	1/2 cup				
Lunch	Beef, broiled	250 g				
	Bread and butter	1 slice				
	Vegetables (2-3 kinds of lettuce, celery, spinach,	150 g				
	potato and carrot), salad or fried	1 cup				
	Soup, containing milk and corn Fruit (banana)	1 Cup				
	Coffee or black tea	1 cup				
Dinner	Beef, broiled or stewed	250 g				
	Bread and butter	1 slice				
	Vegetables (similar to lunch)	150 g				
	Soup (similar to lunch)	1 cup 1				
	Fruit (apple or banana) Ice cream or cake	i				
	Soft drink	1 cup				
	Coffee	1/2 cup				
ttinh Las la	iah maat diat					
Breakfast	igh-meat diet Eggs and bacon or ham, fried	200 g				
Dicuriuse	Bread and butter	1/2 slice				
	Vegetable (lettuce or cucumber), salad	Little				
	Coffee	1/2 cup 330 g				
Lunch	Beef, broiled Bread and butter	1/2 slice				
	Vegetables (potato, carrot and/or onion), salad or	Little				
	fried					
	Coffee or black tea	1 cup				
Dinner	Beef, broiled	350 g				
	Bread and butter	1/2 slic				
	Vegetables (similar to lunch)	Little 1 cup				
	Soup, containing milk and corn Coffee	1/2 cuj				
	LOURA	.,				

Variety of items from Western, Chinese and typical Japanese diets

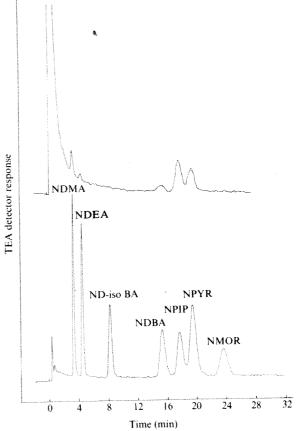


Fig. 1 GLC-TEA (thermal energy analyser) chromatograms obtained for 10 µl dichloromethane extracts of human faeces (upper part) and a standard mixture of volatile nitrosamines (lower part). Fresh faeces were immediately homogenized and weighed: 5 g faecal samples were homogenized in a Teflon homogenizer with 8 g sodium chloride and 20 ml 2 M dosium hydroxide. The samples were then transferred to two 50-ml centrifugation tubes having ground stoppers, and extracted three times with 30 ml dichloromethane (absorbancy analysis reagent; Dōjin) using a shaker. The dichloromethane was separated by centrifugation at 3,000 r.p.m. for 10 min. The extracts were washed with a small volume of distilled water in a separating funnel, and dried over anhydrous sodium sulphate. Sodium sulphate was then washed three times with a small volume of dichloromethane. The combined extracts were evaporated to 0.5 ml in a Kuderna-Danish concentrator at 33 °C for ~1 h. A gas-liquid chromatograph connected to a thermal energy analyser (model TEA-502, Thermo Electron) was used to detect nitrosamines. Glass tubing (2 m× 3 mm) packed with 25% PEG 6,000 on chromosorb W, 60-80 mesh was used in a single-column isothermal gas chromatograph. The carrier gas was argon (flow rate 50 ml min<sup>-1</sup>) and the column temperature was 160 °C. TEA attenuation was ×4. The recovery rates of NDMA, NDEA, NDBA, NPIP, NPYR and N-nitrosomorpholine (NMOR) (added to the faeces at a concentration of 1 mg per kg) were 69, 75, 78, 81, 82 and 81%, respectively. The detection limit was in the range 0.2-0.5 µg per kg.

~2.8 kcal, 25% protein (mostly animal protein), 55% fat, 20% carbohydrate and 70 mg vitamin C. The mixed Japanese diet consists of various food items, including those typical of Western, Chinese and Japanese diets, and is popular among contemporary Japanese. This diet provided ~2.4 kcal, 20% protein, 30% fat, 50% carbohydrate and 90 mg vitamin C. The high-fat, high-meat diet is mostly animal fat and meat with small amounts of bread and vegetables, which provides ~3.0 kcal, 30% protein, 65% fat, 5% carbohydrate and 15 mg vitamin C. Nitrate contents of these diets were estimated according to Walker<sup>16</sup> and Yanagihara et al. To be: typical Japanese diet, 450 mg; balanced Western-style diet, 360 mg; mixed Japanese diet, 250 mg; and high-fat, high-meat diet, 50 mg.

Several volatile nitrosamines were detected in normal human faeces (Fig. 1). The five peaks were identified as N-nitroso-

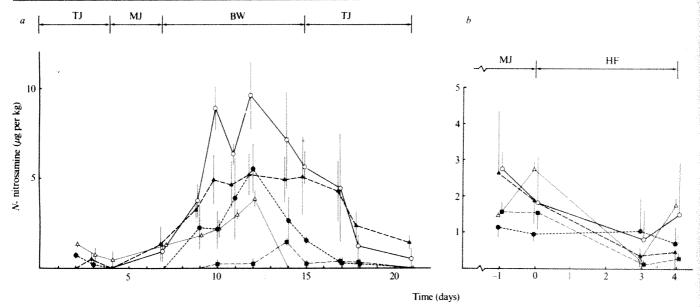


Fig. 2 Changes in levels of volatile nitrosamines in human faeces from individuals on Japanese and Western-style diets (a), and mixed Japanese (MJ) and high-fat, high-meat (HF) diets (b). ●, NDMA; ■, NDEA; △, NDBA; ○, NPIP; ▲, NPYR. TJ, typical Japanese diet; BW, balanced Western-style diet. Values represent the mean ± s.e.m. for three or four subjects.

dimethylamine (NDMA), N-nitrosodiethylamine (NDEA), Nnitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR) by comparison with standards. A control experiment showed that the reagents and glassware used in the analysis did not contaminate the volatile nitrosamines, and that the addition of 1 M sulphamic acid (which decomposes NO<sub>2</sub>), 50 mM ascorbic acid (which inhibits nitrosation of amines<sup>18</sup>) or 0.1 M sodium nitrite (a precursor of nitrosamines) to the faecal samples did not affect the yield of nitrosamines. Furthermore, we analysed the faecal samples immediately after collecting specimens. These experiments indicated that there was no artificial formation of nitrosamines during either the analysis<sup>15</sup> or storage<sup>19</sup>. To extract volatile nitrosamines from faeces using dichloromethane, the distribution ratio must be improved by salting out and alkalization of the faecal samples. Volatile nitrosamines in faeces may exist in a state which is difficult to extract, possibly because the nitrosamines are contained in undigested food, or bound chemically or physically to some compounds.

The changes in levels of volatile nitrosamines in faeces observed for the different diets are shown in Fig. 2. Each subject showed a similar pattern of nitrosamine levels. In the case of the typical Japanese diet, faeces contained NDMA, NDBA and NPYR at levels below 1.7 µg per kg. The amount of faecal nitrosamines detected varied from day to day. After 3 days on the mixed Japanese diet, NPIP appeared and the concentration of NPYR increased. In the case of the balanced Western-style diet, these nitrosamines increased in faeces markedly, particularly NPIP, which reached a maximum of 13.3 µg per kg. The maximum total amount of nitrosamines was 26.6 µg per kg. NDMA, NPIP and NPYR were detected constantly during the study period for the balanced Western-style diet, but no NDBA was detected on the latter days of the diet. After the change from the balanced Western-style diet to the typical Japanese diet, the levels of these nitrosamines in faeces were reduced but not rapidly. NDEA was detected at a maximum of only 1.8 µg per kg during this experimental period, and there was no clear change in amounts of NDEA with change in diet. On the other hand, these nitrosamines decreased in individuals when taken off the mixed Japanese diet and given the high-fat, high-meat diet (Fig. 2b).

These results suggest that volatile nitrosamines in faeces do not originate from nitrosamines formed in fat during cooking<sup>20,21</sup>, but are formed in the intestine from precursors contained both in meat and vegetables. N-nitrosamines may be formed from precursors in the intestine in the following way.

Nitrate, which is largely contained in vegetables<sup>16,17</sup>, is reduced to nitrite by oral or intestinal bacteria<sup>22,23</sup>. Amino acids and lipids, largely contained in meat, are decomposed to secondary amines by the intestinal bacteria<sup>24,25</sup>. Finally, N-nitrosamines are formed from nitrite and secondary amines by the intestinal bacteria in the neutral or weak alkaline conditions of, for example, the lower intestine 26.27. It is considered that the low levels of volatile nitrosamines found in faeces of individuals given the typical Japanese diet or the high-fat, high-meat diet are due to the low amounts of amino acids and lipids in the former diet and the low amounts of nitrate in latter. Sufficient quantities of nitrate and amino acid or lipid, such as in the balanced Western-style diet, will be necessary for the formation of nitrosamines in the intestine. It is known that vitamin C inhibits nitrosation of amines<sup>18</sup>. The vitamin C content in the balanced Western-style diet was nutritionally sufficient, but was insufficient to inhibit nitrosation. The average level of total volatile nitrosamines in faeces in the case of the nutritionally balanced Western-style diet was 10 times that in faeces from those given the typical Japanese diet. This may have strong implications for the aetiology of colon cancer. It is possible that N-nitrosamines in the faeces are responsible for many other kinds of cancer, for example, breast and prostate, as these show the same epidemiological pattern as colon cancer<sup>1,2</sup>. Further investigation of the carcinogenicity of N-nitrosamines, and the inhibition of N-nitrosamine formation in the intestine by the control of diets or intestinal bacteria is needed.

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#### Anti-PGE antibodies inhibit in vivo development of cell-mediated immunity

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Prostaglandins have been implicated in the regulation of humoral and cell-mediated (CMI) immunity, but little is known about their precise mode of action; published data indicate both enhancing and suppressing activity of these substances<sup>1</sup>-E-type prostaglandins (PGEs) act as extracellular modulators of the function of T lymphocytes<sup>5,6</sup> and there may be differences in the sensitivity of different T-cell subpopulations to this modulation<sup>7-9</sup>. As most studies have used in vitro test systems, it is unknown whether prostaglandins have the same effects in vivo4. We have therefore examined the role of PGEs in CMI in vivo. Prostaglandins are derived from essential fatty acids (EFA) via the cyclooxygenase system, and inhibition of this pathway by aspirin or indomethacin<sup>10,11</sup> has been widely used to study prostaglandin effects. However, the usefulness of these substances in vivo is restricted by their toxicity and limitations in their selective action<sup>12,13</sup>. An alternative is to use anti-prostaglandin antibodies, which antagonize F- and E-type prostaglandins in experimental animals 14,15 and prevent PGI<sub>2</sub> from inhibiting ADP-induced platelet aggregation16. We report here that anti-PGE antisera significantly suppressed experimental allergic encephalomyelitis (EAE) in rats and prevented the generation of host-versus-graft (HvG) and graft-versus-host (GvH) reactions in mice, suggesting that PGEs are important mediators not only of in vitro induction of CMI responses<sup>8,9</sup> but also of the early phase of CMI in vivo.

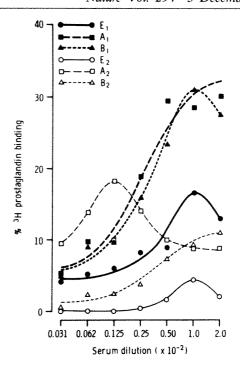


Fig. 1 Radioimmunoassay of an anti-PGE<sub>1</sub> antiserum (APS) raised in Lewis rats by s.c. injection of 0.2 mg of a PGE<sub>1</sub>-BSA conjugate emulsified in complete Freund's adjuvant (CFA) followed by two injections, at monthly intervals, of 0.1 mg of conjugate in CFA. Serum was collected on day 10 after the third inoculation of antigen and thereafter at monthly intervals, 10-12 days after further boosting with  $0.1\,\mathrm{mg}$  of conjugate. A similar protocol was used for the production of APS in rabbits and mice with the doses of antigen adjusted to the different body weights: rabbits received 2.0 and 1.0 mg and mice 0.02 and 0.01 mg of the conjugate. Control sera were raised by the same method but using injections of BSA in CFA only. Anti-PG activity was tested by radioimmunoassay. Serial dilutions of APS were carried out in V-shaped microtitre plates (Sterilin). 3H-labelled prostaglandins  $E_1$ ,  $A_1$ ,  $B_1$  and  $E_2$ ,  $A_2$  and  $B_2$  (specific activity 50 Ci mmol<sup>-1</sup>; Amersham) were added to the wells at an activity of  $\sim 12,000$  counts per min. After incubation for 1 h at 37 °C, 20 µl of anti-rat IgG (H+L; Nordic Immunology Laboratories, UK) and 15  $\mu$ l of normal rat serum (diluted 1:100) were added. After further incubation for 16 h at 4 °C the plates were centrifuged (500g at 4 °C for 30 min). The supernatants were decanted and the precipitates dissolved in 0.2 ml of 0.1M NaOH and transferred to 2 ml NE260 scintillation fluid (Nuclear Enterprises), Radioactivity was measured in a liquid scintillation counter. All tests were carried out in triplicate. A background activity of 5-6% was measured in the control serum as well as in normal rat serum, and this activity was subtracted from that found for APS.

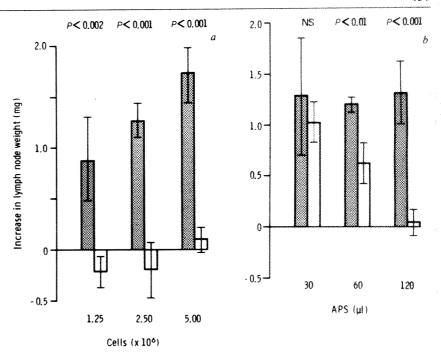
Anti-prostaglandin antiserum (APS) was raised by repeated subcutaneous (s.c.) injections in Lewis rats, NZW rabbits or CBA mice of a conjugate of PGE<sub>1</sub> and bovine serum albumin (BSA) emulsified in complete Freund's adjuvant<sup>17,18</sup>. Antiprostaglandin activity of these sera compared well with those reported elsewhere 16-19. Figure 1 shows the anti-PGE<sub>1</sub> activity

Table 1 Suppression of EAE in Lewis rats by antisera containing antibodies to PGEs

		No. of rats per			EAI	E prevalence on	various days af	ter antigen inoc	ulation	
Expt	Group	group	Treatment	Day 13	Day 15	Day 17	Day 19	Day 21	Day 23	Day 29
1	а	6	Control serum	0 (0)	2 (3)	6 (20)	6 (17)	6 (17)	6 (18)	6 (17)
	b	6	APS	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	2 (8)	2 (8)
2	а	8	Control serum	2 (2)	5 (7)	7 (20)	8 (34)	8 (30)	6 (22)	4 (8)
	ь	8	APS	0 (0)	0 (0)	0 (0)	1 (1)	1 (6)	1 (6)	2 (8)
Difference	in EAE prev	alence between	groups $a$ and $b^*$	NS	$P \leq 0.01$	P < 0.002	P < 0.002	P < 0.002	P < 0.002	NS

EAE was produced by s.c. injection of guinea pig brain stem and spinal cord material emulsified in complete Freund's adjuvant<sup>21,22</sup>, and severity of clinical disease scored in the range 0 (= no disease) to 8 (= death due to EAE)<sup>21,22</sup>. The batches of control serum and APS used were raised in Lewis rats (expt 1) or NZW rabbits (expt 2). APS or control serum (0.25-0.30 ml) was injected three times weekly between day 0 and day 21 (expt 1) or day 23 (expt 2). After discontinuation of the treatment, a few more animals in the two APS groups developed EAE, but no further increase in disease prevalence was observed after day 29 (for a further observation period of  $\sim$ 2 months). EAE prevalence = no. of rats per group showing clinical signs of EAE at any one of the days indicated; the sum of clinical scores per group is given in parentheses Significance by Fisher's exact test for experiments 1 and 2 pooled. NS, not sigificant.

Fig. 2 The effect of APS on HvG reaction in CBA mice. CBA mice were inoculated in the left footpad with irradiated (2,000 rad) spleen cells of A strain mice, and the right footpad was injected with irradiated spleen cells from CBA mice. The extent of the resulting reactions was determined by measuring the increase in weight of the left popliteal lymph node over the weight of the right node. a The weights of the popliteal lymph nodes were measured on day 3 after inoculation of the mice with various concentrations of irradiated spleen cells. APS or control serum (derived from CBA mice) was injected s.c. into the nuchal region at a dose of 30 µl per day. The first injection was on day 0, immediately after inoculation of the cells (time 0), and was followed by further injections on days 1 and 2. b, APS or control serum (derived from CBA mice) was given as a single s.c. injection on day 0 immediately after footpad inoculation of CBA mice with a fixed number (5×106) of spleen cells. Columns represent the mean increase in weight (±s.d.) of the left popliteal lymph nodes over the weight of the right popliteal nodes in groups of six mice. Shaded and open columns represent treatment with control serum and APS, respectively. The differences between control groups and APS-treated animals were assessed using Student's t-test. NS, not significant.



of a batch of APS used to treat EAE. Dehydration and rearrangement of double bonds can result in the conversion of some of the PGE<sub>1</sub> into PGA<sub>1</sub> and PGB<sub>1</sub> during the conjugation of PGE<sub>1</sub> to BSA. A similar conversion may also occur after injection of the conjugate, due to the action of the recipients' dehydrating and isomerizing enzymes<sup>20</sup>. Thus, not only did we detect anti-PGE<sub>1</sub> activity but also activities against PGA<sub>1</sub> and PGB<sub>1</sub>, as well as cross-reactivity with PGE<sub>2</sub> and its respective derivatives (Fig. 1). As we were less successful in raising APS by injection of a PGE<sub>2</sub>-BSA conjugate, all experiments reported here used only serum produced by injections of PGE<sub>1</sub>-BSA. Control sera were raised by injection of BSA emulsified in complete Freund's adjuvant, using the same method as for production of anti-PG antiserum.

We first tested the effect of APS on EAE. This CMI disease was induced in Lewis rats and its activity determined as described previously<sup>21,22</sup>. Treatment with s.c. injections of APS from the day of antigen inoculation (day 0) up to days 21-23 suppressed EAE (see Table 1). Not only were there significantly fewer animals developing clinical signs of EAE but also onset of the disease was delayed in the APS-treated groups.

As the use of EAE in rats was too complicated for investigating the dose-response effect of APS and its inhibitory action in relation to the time of administration, we used mice in further experiments. HvG and GvH reactions induced by injection of allogeneic cells or cells of parental strain animals were measured in a lymph node assay system. We found that APS treatment significantly suppressed both HvG and GvH reactions in mice. Injections s.c. of 30 µl APS per day, from day 0 to day 2 after challenge of CBA mice with A strain spleen cells almost completely inhibited the HvG reaction (Fig. 2a). The inhibitory effect of a single dose of APS given immediately after the injection of donor cells was dose dependent in HvG (Fig. 2b) as well as in GvH experiments (Fig. 3). GvH reactions were reduced to about half by 20-30 µl APS; 60 µl were needed to achieve a similar effect in HvG reactions. Studies of the effect of APS in relation to time of its administration showed that the sera were most effective during the first hours after challenge of the animals with donor cells (Fig. 4). This was most pronounced in HvG reactions: the inhibitory effect of a single dose of APS (120 µl) given at time 0 was much greater than the effect of APS given 6 h after the injection of donor cells. In GvH reactions an inhibitory effect was still observed when APS was administered as late as 24 h after cell injection. However, inhibition was again greatest during the first hours after the inoculation of parental strain cells.

PGEs have been found to inhibit mitogen-induced lymphocyte proliferation<sup>23-25</sup>, lymphocyte-mediated cytolysis<sup>26</sup> and the production of lymphokines<sup>5,6,27</sup>. Treatment in vivo with PGE<sub>1</sub> prevented development of anaemia and progression of nephritis in murine lupus erythematosus<sup>28,29</sup> and the development of hypersensitivity responses was reduced in mice<sup>30</sup>. Furthermore, PGE<sub>2</sub> restricted lymphocyte traffic through antigen-stimulated lymph nodes, possibly by modulating the interaction of the lymphocytes with adherent cells within the nodes<sup>31</sup>. In two recent studies in vitro<sup>8,9</sup>, PGEs affected the induction phase of one-way mixed lymphocyte reactions and development of lymphocyte cytotoxicity. Cell proliferation as well as cytotoxicity were enhanced when the cultures were treated with indomethacin, but the response was restored to normal by

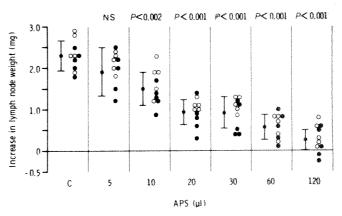


Fig. 3 Dose response of APS in a GvH reaction. (CBA×C57BL/10)F₁ mice received spleen cells from C57BL/10 mice in the left footpad and spleen cells from (CBA×C57BL/10)F₁ animals in the right. Various doses of APS were injected s.c. into the nuchal region on day 0, immediately after the footpad inoculation of 5×10<sup>6</sup> spleen cells. The weights of the popliteal lymph nodes were measured 4 days after inoculation of the cells. Data points show increase in weight of the left popliteal node in individual animals treated with APS or control serum (C) as derived from CBA mice (○) or NZW rabbits (●). Bars represent the mean increase in weight (±s.d.) per group. (For statistical evaluation see Fig. 2 legend.)

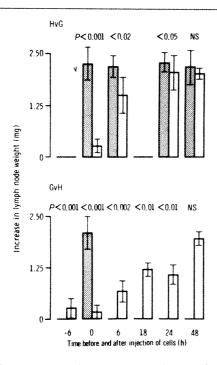


Fig. 4 The effect of a single dose of APS on HvG and GvH reactions in mice, in relation to the time of APS administration. Groups of CBA and (CBA×C57BL/10)F<sub>1</sub> mice (five per group) received footpad inoculations of  $5 \times 10^6$  donor spleen cells; APS was injected at different time intervals before or after cell inoculation. Lymph node weights were determined on day 3 (HvG) or day 4 (GvH) after cell inoculation. Columns represent the mean increase in weight (±s.d.) per group of the left popliteal lymph nodes in animals treated with APS (open columns) or control serum (shaded columns). (For statistical evaluation see Fig. 2 legend.)

adding PGE2 to the cultures. Thus, it was suggested that PGEs may be negative (that is, inhibiting) mediators of CMI development.

In contrast to these in vitro findings, our in vivo data indicate that PGE is required as a positive (augmentative) mediator substance during the induction phase of CMI. These contrasting results could be reconciled if the effect of prostaglandin was governed by a bell-shaped dose-response relationship. According to our hypothesis, low prostaglandin concentrations would be required to initiate or enhance the induction of CMI responses whereas high concentrations would inhibit this process. Anti-PG antibodies may be more effective than indomethacin in reducing the concentrations of active prostaglandin at the site of action and treatment with such antibodies might thus deprive the immunological microenvironment of agents required for the induction of immune responses.

Our results emphasize the potential importance of anti-PG antibodies as tools for elucidating the effects of different prostaglandins and other EFA derivatives on immune mechanisms. It might also be possible to use such antibodies to correct immunological disorders. However, a shortcoming of the APS used here is its polyvalent nature. Greater specificity could be achieved using monoclonal antibodies, and experiments to explore this possibility are in progress.

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#### Identification of antigenic determinants unique to the surfaces of cells transformed by Epstein-Barr virus

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Epstein-Barr virus (EBV) transforms human B lymphocytes in vitro and is associated with two human lymphoproliferative diseases, Burkitt's lymphoma and infectious mononucleosis. In contrast to the lymphoma, infectious mononucleosis is selflimiting and the finding by Svedmyr and Jondal of cytotoxic T lymphocytes specific for EBV-transformed cell lines in mononucleosis patients' blood during the acute phase suggests that this self-limitation results, in part, from the cell-mediated immune response of the host. These workers termed the structure expressed on EBV-transformed cells and which was recognized by specific, cytotoxic T cells, LYDMA (lymphocyte determined membrane antigen). It has also been shown that with appropriate education in culture, cytotoxic T cells from healthy donors specifically kill autochthonous, EBV-transformed cell lines2. These target cells generally do not produce virus and bear no virion-associated antigens. We have now isolated monoclonal antibodies which detect antigenic determinants unique to the surfaces of EBV-transformed cells but are not associated with virus production. Quantitative assays for these determinants indicate that they are expressed strongly on cells transformed in vitro by EBV but are expressed at intermediate or undetectable levels on cell lines established from patients with Burkitt's lymphoma. These antigenic determinants may be among those recognized by autochthonous, cytotoxic T cells specific for EBV-transformed cell lines, and if they are, their diminished expression on tumour-derived cells may reflect a selective advantage in vivo for those tumour cells.

Spleen cells from C57BL/6 or BALB/c mice immunized by repeated intraperitoneal injections of 107 cells of one clone of EBV-transformed human cells were used to generate hybridomas following the method of Kohler and Milstein3. A twostep screening was performed to select those hybridomas whose antibodies bound to an EBV-transformed cell clone different from that used for immunizations but not to primary T lymphocytes. Eighteen hybridomas which showed this specificity of binding were isolated, cloned at least three times and injected into pristane-primed mice to form ascites tumours.

The monoclonal antibodies from the 18 different ascitic fluids were first characterized by assaying for their binding to a panel

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Table 1 Binding of monoclonal antibodies to human cell lines

Binding	Description of cell line	Association with EBV	Ref.
Strongly positive	Five clones of cells transformed in vitro by EBV from three donors	+	10
	Four cell lines transformed in vivo and not associated with Burkitt's lymphoma: WI-L2, RPMI 1788, E.H. IV, CCRF-SB	+	11, 12, 13, 12
Intermediate	Four cell lines established from four Burkitt's lymphoma patients: B35M, B46M, AG876, Raji	+	12, 12, 14, 12
Negative	Three cell lines established from three Burkitt's lymphoma patients: EB-3, Daudi, P3HR-1	+	15, 12, 16
regative	One EBV-negative Burkitt's lymphoma cell line, BJA-B-1	····	16
	One acute lymphocytic B-cell line, BALL-1	regue	12
	One multiple myeloma cell line, RPMI 8226	***	12
	One lymphosarcoma B-cell line, U-698-M	-	12
	One acute lymphocytic T-cell line, CCRF-CEM	-	12
	One acute lymphocytic T-cell line, CCRF-HSB-2	***	12
	One erythroleukaemia cell line, K-562	need	12
	One chronic myelocytic non-B, non-T cell line, NALM-1	MARY	12
	One human fibroblast strain	2007	

The binding of the four monoclonal antibodies to human cell lines was measured with a sheep red blood cell (SRBC) rosette assay using ascites fluid prepared from each hybridoma and diluted from  $10^{-1}$  to  $10^{-6}$  in 10-fold increments<sup>17</sup>. Aliquots ( $50~\mu$ l) of each dilution were incubated with  $2\times10^5$  cells at  $4^{\circ}$ C in assay buffer: phosphate-buffered saline, 5.0% fetal calf serum and 0.4 mg ml<sup>-1</sup> pooled human  $\gamma$  globulin. After 0.5 h, cells were washed twice and pelleted with  $5\times10^7$  SRBC coupled to affinity-purified goat anti-mouse immunoglobulin. After 0.5 h, the cell pellets were resuspended in  $50~\mu$ l of assay buffer and viewed by light microscopy for rosette formation. The results obtained for binding were classified into three groups by the following criteria: strongly positive =90% of the test eells formed rosettes down to dilutions of  $10^{-4}$ - $10^{-5}$ ; intermediate binding =10-30% test cells formed rosettes down to dilutions of  $10^{-3}$ - $10^{-4}$ ; negative binding =10-rosettes formed at dilutions  $>10^{-1}$ .

of human cell lines. Four of these antibodies have the same specificities. The data in Table 1 indicate that only cell lines transformed by EBV bind these four monoclonal antibodies; cell lines transformed in vitro or those transformed in vivo but not associated with Burkitt's lymphoma bind them strongly; cell lines from patients with Burkitt's lymphoma bind them at intermediate or undetectable levels. Among the cell lines which also fail to bind these antibodies are four derived from B-cell tumours which are not associated with EBV.

Binding of these four antibodies to several of the lines listed in Table 1 was measured quantitatively using a fluorescence-activated cell sorter and the results showed that in identical conditions 85–97% of cells in the strongly positive populations bound the antibodies, compared with 10–30% and 2–5% of the intermediate and negative populations, respectively. In addition, 2–5% of the negative populations bound an anti-H-2K<sup>k</sup> monoclonal antibody which serves as a background for nonspecific binding in this assay. Among the fraction of cells scored as binding, the median fluorescent cell in the strongly positive populations bound five times as much label as did the median fluorescent cell in the intermediate populations. Monoclonal antibodies with the above binding specificity will be termed anti-EBVCS.

EBVCS determinants are not associated with virus production and are therefore different from the virus-associated cell-surface antigen, MA<sup>4</sup>. The cell line, P3HR1, in which ~5% of the cells permit viral maturation and express MA, does not express EBVCS determinants, nor does the B95-8 cell line in which 4-8% of the cells permit viral maturation and express MA. These two cell lines were negative for EBVCS determinants using both a rosetting assay and a direct immunofluorescence assay (Table 1 and unpublished data). In addition, clones of cells transformed *in vitro* by EBV in which fewer than 3 cells per 10<sup>5</sup> release virus per 24 h (ref. 5) are uniformly positive for expression of EBVCS (Table 1).

To determine whether EBVCS determinants are present on or can be induced in primary human lymphocytes, we have assayed for their expression on untreated lymphocytes and on lymphocytes exposed to EBV, concanavalin A (Con A) and pokeweed mitogen (PWM). Between 0.5 and 4% of freshly isolated human lymphocytes bind anti-EBVCS antibodies as well as an anti-H-2K<sup>k</sup> monoclonal antibody. Primary lymphocytes lose this initial background of binding after several days in culture. The data in Fig. 1 indicate that only lymphocytes exposed to EBV express EBVCS determinants, detectable within 48 h after infection. Neither blast transformation induced by Con A nor blast transformation and immunoglobulin secretion induced by PWM in lymphocytes<sup>6</sup> in the absence of

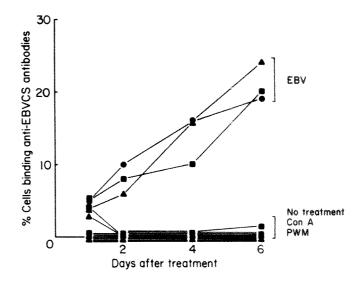


Fig. 1 Lymphocytes isolated from the peripheral blood of healthy donors were infected with the B95-8 strain of EBV at an MOI 1, or were treated with PWM and Con A at concentrations of  $10~\mu g\,ml^{-1}$  and  $2~\mu g\,ml^{-1}$ , respectively. All cells were cultured in RPMI 1640 medium + 10% fetal calf serum. On 1, 2, 4 and 6 days after treatment, a sample of cells from each culture was assayed for the binding of anti-EBVCS antibodies (IgG1A (A), IgG1B (■) and IgM (●)) using an indirect immunofluorescence assay. For each assay,  $4\times10^5$  cells were incubated at  $4\,^\circ\text{C}$  with a  $10^{-3}$  dilution of a hybridoma's ascites fluid in  $100\,\mu\text{l}$  of assay buffer (described in Table 1 legend). After 0.5 h, the cells were washed, incubated with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse immunoglobulin at 4 °C for 0.5 h, washed again and dried on glass slides. Cells were fixed with methanol, counterstained and then scored under UV epifluorescence microscopy. Ascites fluid raised from an anti- $H-2K^k$  hybridoma was used to measure nonspecific binding. A cloned line of in vitro-transformed cells (THLB1) was included in each assay as a positive control and gave ≥90% fluorescent staining cells. For each point, 100-300 cells were counted and the data were expressed as the per cent of positive staining cells. The immunoglobulin subclass of the four anti-EBVCS antibodies was determined using FITCgoat anti-mouse IgG1, IgG2 and IgM. Although all four EBVCS antibodies vere used, results are given for the three which were isolated from separate fusions. The results for the fourth anti-EBVCS, which is an IgM antibody and isolated from the same fusion as the other IgM, are equivalent to those shown. The mitogenic response of primary lymphocytes to Con A and PWM treatment was established by measuring stimulation of DNA synthesis.  $10^6$  cells from each culture were pulsed with  $0.5\,\mu\text{Ci}^{-3}\text{H-thymidine}$ 1) in 1.0 ml for 24 h beginning on day 3. Cells were lysed and (50 Ci m mol incorporated <sup>3</sup>H-thymidine was determined by trichloroacetic acid precipitation. Fourfold more <sup>3</sup>H-thymidine was incorporated into the Con Aand EBV-treated cultures than into the untreated culture. The per cent of cells expressing EBNA in the untreated and EBV-infected cultures was assayed as published previously and was found to be 0.0% and 10%, respectively.

Table 2 EBNA-positive and EBVCS-positive cells form overlapping populations

Antibody used in assay	Unseparated lymphocytes (%)	T cells (%)	B cells (%)
Anti-EBNA	14	1.5	66
Anti-H-2K <sup>k</sup>	0	0	3.3
Anti-EBVCS-IgG1A	16	0.5	63
Anti-EBVCS-IgG1B	11	0.5	63
Anti-EBVCS-IgM	12	1	62

After 5 days in culture, a portion of EBV-infected primary lymphocytes was left unseparated and a portion was separated into T- and B-cell populations using Petri dishes coated with rabbit anti-human Fab antibodies8. These three populations of cells were scored for EBVCS determinants by indirect immunofluorescence as described in Fig. 1 legend and for EBNA by anti-complement immunofluorescence<sup>7</sup>, 100-300 cells were counted for each assay and the data expressed as the per cent of cells exhibiting immunofluorescence. THLB1 cells were included in each assay and ≥90% of the cells stained positively for both EBNA and EBVCS

EBV leads to detectable expression of EBVCS determinants. The observed association between transformation by EBV and expression of EBVCS determinants indicates that those infected cells which develop expression of EBNA7, the EBVinduced nuclear antigen, may also be those which express EBVCS. To test this prediction, primary lymphocytes were exposed to EBV, cultured for 5 days and a portion of the cells separated into immunoglobulin-positive and -negative populations by binding them to plastic dishes coated with rabbit anti-human Fab antibodies8. Assay of unseparated populations and the immunoglobulin-positive and -negative populations (Table 2) indicates that those cells which express EBVCS determinants are separated as B cells along with those which express EBNA and that the two antigen-expressing populations are overlapping and may be identical.

To identify the molecule(s) which carries the EBVCS determinants, cells were labelled with 125 I using lactoperoxidase, and immunoprecipitates of extracts of the labelled cells were resolved on polyacrylamide gels. The results depicted in Fig. 2 indicate that one of the two IgG class antibodies precipitates a polypeptide with an apparent molecular weight of 45,000 from only the EBVCS-positive cell line. Although this polypeptide is

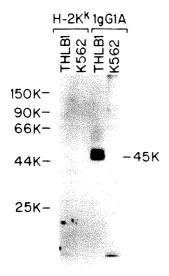


Fig. 2 Immunoprecipitations using monoclonal antibodies were carried out on lysates prepared from  $2\times10^7$  THLB1 (EBVCS positive) and  $2\times10^7$  K562 (EBVCS negative) cells labelled with <sup>125</sup>I and lactoperoxidase as described previously <sup>18</sup> with the following modification: 2.5  $\mu$ I of ascites fluid from either the IgG1A (anti-EBVCS) or anti-H-2K<sup>k</sup> and 2.0  $\mu$ I of affinitypurified goat anti-mouse immunoglobulin (6.0 mg ml<sup>-1</sup>) were used for each precipitation before addition of formalin-fixed Staphylococcus aureus. Immunoprecipitated proteins were electrophoresed in an SDS-polyacrylamide gel and visualized using autoradiography as described elsewhere . The molecular weight markers were identified by staining the gel with Coumassie blue before preparing it for autoradiography. The markers consist of *Escherichia coli* RNA polymerase (150 K, 90 K), bovine serum albumin (66 K), ovalbumin (44 K) and chymotrypsinogen (25 K).

likely to be the molecule which carries EBVCS determinants, its certain identity awaits further experimentation.

Four monoclonal antibodies bind to the same spectrum of cells and identify EBVCS determinants unique to EBV-transformed cells. The use of these monoclonal antibodies to assay these determinants quantitatively indicates that EBVCS determinants are expressed at reduced levels on cell lines established from Burkitt's lymphoma patients relative to their expression on all other EBV-transformed cell lines tested. The EBVCS determinants are among possible determinants recognized by autochthonous, cytotoxic T cells specific for EBVtransformed cells and may therefore be equivalent to LYDMA. The possibility of this equivalence is supported by the finding that EBVCS and LYDMA are expressed with the same time course in lymphocytes infected with EBV in vitro (Fig. 1 and ref. 9). If the EBVCS determinants are used by cytotoxic T cells to identify their targets, the reduced expression of EBVCS on Burkitt's lymphoma cell lines is consistent with these tumour cells having been selected in vivo to survive that cytotoxic response.

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#### **Specificity of T-cell clones** illustrates altered self hypothesis

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Cytotoxic T lymphocytes (CTLs) recognize foreign antigens in the context of self major histocompatibility antigens. In the mouse this phenomenon is called H-2 restriction as H-2 is the major histocompatibility complex. Two theories have been proposed to explain the dual requirement for CTLs to kill specifically target cells: (1) the two-receptor hypothesis proposes that T cells have an anti-self H-2 receptor and a distinct anti-foreign (anti-X) receptor<sup>1,2</sup>; (2) the altered self hypothesis proposes that T cells recognize complex antigens or interaction antigens created by the physical interaction of self H-2 molecules and X in the target cell membrane<sup>3,4</sup>. Here we report the isolation of CTL clones which recognize H-2k-plus-X and crossreact with H-2<sup>d</sup>-plus-Y. Because the clones recognize two pairs of antigens and not any combination, for example, H-2k-plus-Y, they support very strongly the principle of altered self recognition, that is, that only the complex of H-2 and foreign antigen is recognized and not either component independently.

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In one set of experiments we have isolated a number of cloned, diploid, H-2k/H-2d CTL lines which were originally selected in vitro to recognize H-2k-plus-minor antigen 1 but which also recognize H-2<sup>d</sup>-plus-minor antigen 2. Radiation chimaeras (F<sub>1</sub> + parent) were constructed by reconstituting lethally irradiated B10.D2 (H-2<sup>d</sup>) mice with (B10.BR× B10.D2) $F_1$  H-2<sup>k</sup>/H-2<sup>d</sup> bone marrow cells. When immunized in vivo and in vitro with minor H antigen-different H-2k/H-2d  $(BALB.K \times BALB/c)F_1$  cells, these chimaera cells show a 10-to >50-fold preference for reacting to H-2<sup>d</sup> (that is, BALB/c) target cells. Therefore, as has been shown previously<sup>5-7</sup>, these chimaeras have a self or thymic preference in H-2 restriction. The H-2k-restricted CTLs from one chimaera which had a 20-fold H-2<sup>d</sup>-restriction preference were selected by stimulation in mixed lymphocyte culture (MLC) with irradiated BALB.K cells on days 0 and 9 of culture. On day 14 these cultures reacted well with BALB.K targets but significantly also

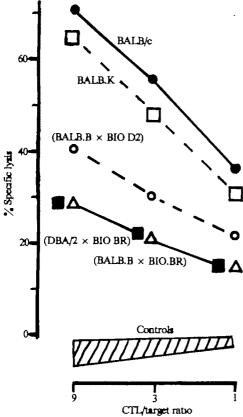


Fig. 1 Cytotoxic activity of CTL clone cr-15 on various labelled target cells. A radiation chimaera, (B10.BR×B10.D2)→B10.D2, was primed in vivo 8 weeks following the irradiation by an injection of  $(BALB.K \times BALB/c)F_1$  spleen cells. After 8 weeks, spleen and lymph node cells from the chimaera were placed in MLC with irradiated BALB.K spleen cells as stimulators in MLC medium Cells were stimulated once more with BALB.K stimulators and twice with BALB/c stimulators and were cloned on day 40 of culture according to the technique of Glasebrook and Fitch<sup>16</sup>. Long-term MLC cells were suspended at 3 cells ml<sup>-1</sup> with irradiated BALB/c spleen cells (3×10<sup>6</sup> cells ml<sup>-1</sup>) in MLC medium containing 40% conditioned medium from concanavalin Aactivated mouse spleen cells plus 50 mM a-methylmannoside. Aliquots (0.1 ml) were cultured in the wells of Coster 3596 plates. Cytotoxic ciones were maintained by weekly subculture in conditioned medium plus irradiated stimulator cells. Five months after the initial cloning, cr-15 CTLs were assayed for lysis of 51 Crlabelled spleen cell targets which had been cultured for 3 days with lipopolysaccharide. Targets were: BALB/c (●), BALB.K (□),  $(BALB.B \times B10.D2)F_1$ (O), (BALB.B×B10.BR)F<sub>1</sub> (DBA/2×B10.BR)F<sub>1</sub> (Δ). Control targets which were not sensitive to lysis included B10.D2, B10.BR, BALB.B, DBA/2 and  $(DBA/2 \times B10.D2)F_1$ . Six independent cr clones isolated at the same time as cr-15 were also able to kill both BALB/c and BALB.K targets. Furthermore, all four subclones of cr-15 had the same specificity.

reacted strongly to BALB/c targets. The cultured cells were subsequently stimulated in MLC with irradiated BALB/c stimulator cells on days 19 and 31 and were eventually cloned in limiting dilution (see Fig. 1 legend). Seven CTL clones (and subsequently a number of subclones) all with the same cytotoxic specificity were isolated. (The derivation of these clones will be described in detail elsewhere.) Figure 1 shows that the (B10.BR×B10.D2)F<sub>1</sub> CTL clone, cr-15, lyses BALB/c (H-2<sup>a</sup>) and BALB.K (H-2k) target cells very efficiently, but does not lyse either syngeneic (B10.BR×B10.D2)F<sub>1</sub> target cells nor target cells from either parent. That the CTL activity measured on BALB targets is actually true H-2-restricted anti-BALB minor activity is shown by the following cases of 'F1-specific lysis'. BALB.B (H-2b), B10.BR and B10.D2 targets are not lysed, but target cells from (BALB.B×B10.BR)F1 and (BALB.B×B10.D2)F<sub>1</sub> mice are sensitive to lysis. In these F<sub>1</sub> targets, the BALB.B parent provides a minor H antigen and the B10.BR or B10.D2 parent provides the correct restricting H-2 allele.

The most important question regarding the specificity of these CTL clones is whether the  $H-2^k$ -restricted and  $H-2^d$ -restricted minor H antigens recognized are the same or different. Clearly both antigens are present in the BALB non-H-2 background, but the B10 and BALB strains differ by >40 minor H alleles<sup>2,9</sup>. We have been able to separate the antigens genetically to show that in fact the  $H-2^k$ - and  $H-2^d$ -restricted antigens are different: cells from the DBA/2 ( $H-2^d$ ) strain and (DBA/2×B10.D2)F<sub>1</sub> mice are not sensitive to lysis by these CTL clones, whereas (DBA/2×B10.BR)F<sub>1</sub> target cells are lysed . Thus, we conclude that the  $H-2^d$ -restricted minor H antigen is present in BALB but not in DBA, whereas the  $H-2^k$ -restricted antigen is present in both BALB and DBA mice.

Further tests with these clones (Table 1) suggest that the  $H-2^k$  restriction maps to  $K^k$  as C3H ( $K^kD^k$ ) targets are sensitive to lysis whereas C3H.OH( $K^dD^k$ ) targets are not. The  $H-2^d$  restriction of the cr CTL clones probably maps to  $D^d$  as BALB/c ( $K^dD^d$ ) targets are sensitive to lysis while BALB.B ( $K^bD^b$ ) and BALB.HTG ( $K^dD^b$ ) targets are not. We conclude that these CTL clones recognize minor antigen 1-plus- $K^k$  and minor antigen 2-plus- $D^d$ .

The binding specificity of CTLs can also be studied in cold target competition assays in which the release of <sup>51</sup>Cr from one target is studied in the presence of an excess of unlabelled competitor cells. For CTL clone cr-15, the rate of lysis of <sup>51</sup>Cr-labelled BALB/c or BALB.K targets is inhibited by either BALB/c or BALB.K competitors (Table 2). This result reaffirms the clonality of the CTLs and suggests that the two targets cells interact with the same receptor. Unlabelled DBA/2 and BALB.B targets do not inhibit the CTLs. We consistently found that cold BALB/c cells are more efficient competitors than cold BALB.K even when BALB.K is the labelled indicator cell. With <sup>51</sup>Cr-labelled BALB/c as the indicator cell, BALB.K cells are 5–10-fold less efficient inhibitors than BALB/c cells. Thus, the CTLs seem to have a higher affinity for the H-2<sup>d</sup> target.

Table 1 Cytotoxic specificity of cr-15 CTLs

LPS blast target	K H	I-2 allel	es D		fic lysis at et ratio of: 2.3:1
(B10.BR×B10.D2)F <sub>1</sub> BALB/c BALB.K BALB.B BALB.HTG C3HeB/FeJ C3H.OH	k/d d k b d k	k/d d k b d k	k/d d k b k k	-2 64 62 -2 -3 71 -4	0 51 46 0 0 53

Cloned cr-15 CTLs were assayed for lysis of <sup>51</sup>Cr-labelled lipopoly-saccharide (LPS)-induced spleen cells derived from various strains of mice

Table 2 Cold target inhibition studies of the cr-15 cloned CTL line

Unlabelled cells	% Specific 51Cr release from:			
added	BALB/c	BALB.K		
None	35	26		
BALB.B	35	28		
DBA/2	37	26		
$(B10.BR \times B10.D2)F_1$	36	27		
BALB/c	2	-2		
BALB.K	16	0		

<sup>51</sup>Cr-labelled target cells (4×10<sup>4</sup>) of BALB/c or BALB.K origin were mixed with  $2.7\times10^4$  cloned cr-15 CTLs in 1 ml of assay medium in the presence or absence of  $1.8\times10^6$  unlabelled cells from the various strains shown. The cytotoxic assay was incubated for 3.5 h. Labelled and unlabelled target cells were spleen cells cultured for 3 days with a mitogenic dose of lipopolysaccharide.

We have isolated other CTL lines having a similar crossreactive specificity. For example, an in vitro line of (C3H× DBA/2)F<sub>1</sub> (H-2<sup>k</sup>/H-2<sup>d</sup>) CTLs from normal mice binds to and lyses target cells expressing B10 minor antigen-plus-H-2<sup>d</sup> (that is, B10.D2 cells) and trinitrophenyl-bovine serum albumin (TNP-BSA)-coupled C3H (H-2<sup>k</sup>) cells<sup>10,11</sup> but does not recognize either B10 minor-plus-H-2<sup>k</sup> or TNP-BSA-plus-H-2<sup>d</sup> (ref. 12).

The fact that CTLs recognize X-plus-K and Y-plus-D but not the other combinations (X-plus-D, Y-plus-K, X-plus-Y or K-plus-D) shows that H-2-restricted T cells do not recognize foreign antigen and self H-2 as separate entities. The crossreactivity pattern cannot be explained by cross-reactivity at either the anti-self or anti-X sites which have been postulated to exist in two receptor models. The T cells are specific for the complex antigen<sup>13</sup>. Our results agree with those of Kappler et who fused two H-2-restricted cells but did not detect reshuffling of the anti-self and anti-foreign antigen specificities. It seems highly likely that the principle of the altered self hypothesis is correct, that is, self H-2 antigens modify the antigenicity of foreign antigens<sup>3,13</sup>. In this way, CTLs will recognize antigen only when it is associated with the plasma membrane of target or stimulator cells. The probable function of H-2 restriction is to focus the attention of T cells on to membrane-bound antigens.

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#### Multiple conductance states of single acetylcholine receptor channels in embryonic muscle cells

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Acetylcholine (ACh) activates in the synaptic membrane of skeletal muscle an inward current composed of many elementary currents<sup>1,2</sup>. High resolution current measurements in adult frog muscle have shown that the elementary current is a pulselike event of unit amplitude, indicating that ACh opens ion channels which have only two conductance states, fully open or closed3. We now present evidence for a third conductance state. In the membrane of uninnervated embryonic rat muscle we observe that ACh activates two independent classes of currents of different amplitude and average duration, apparently arising from two populations of ACh receptor (AChR) channels. The currents from both classes show, at low incidence, transitions between a main level and a sublevel of lower amplitude. From this we conclude that AChR channels in embryonic muscle adopt, in addition to a 'main' conductance state, a 'substate' of lower conductance.

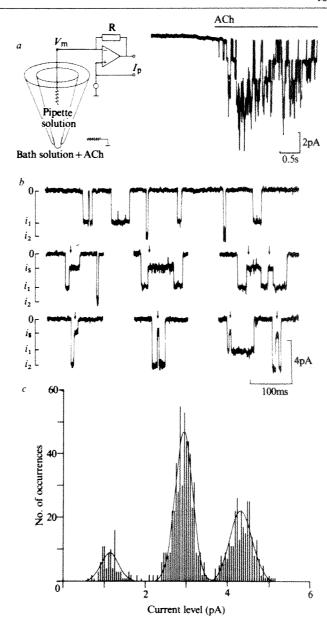
Single channel currents activated by ACh were recorded from cell-free membrane patches isolated from spherical 'myoballs'. Most experiments were performed at low temperatures (5-8 °C) using the 'outside-out' patch configuration4, in which the extracellular membrane face is exposed to the bath solution (Fig. 1a). Following addition of ACh to the bath solution at 1-2  $\mu$ M the membrane current increases from the resting level in a stepwise fashion by superposition of elementary current contributions (Fig. 1a). Figure 1b shows the waveforms of individual current contributions recorded from one patch at -100 mV. They occur predominantly as rectangular pulses of current of two different amplitudes (Fig. 1b, upper record). The mean amplitudes of these 'main' current levels are 4.3 pA and 2.9 pA, respectively (Fig. 1c). A small percentage ( $\leq 10\%$ ) of currents, however, has a more complex waveform, representative examples of which are shown in the middle and lower records of Fig. 1b. The membrane current jumps from either of the two main levels to a smaller 'sublevel' of 1.1 pA average size.

The amplitude and duration of the sublevel were measured from that class of currents where the main level was of the lower amplitude, because in all patches examined this is the more frequently occurring event. In the patch described above where the larger currents made up nearly 40% of all recorded currents, comparison of the sublevel amplitudes of the two classes (Fig. 1b, lower trace) showed that they were not significantly different, both having averages of 1.1 pA. This indicates that the size of the sublevel does not depend on the preceding main level. When the complex current waveforms described above were examined at higher time resolution (4 kHz bandwidth) no transition of the current to the baseline was seen between the two levels. At this time resolution the coincidental occurrence of two individual currents within 0.5 ms would have been detected as separate events. Following the initial transition from the main level to the sublevel the current jumps either back to the resting level or switches between the sublevel and the main level (Fig. 1b, middle traces). The frequency of occurrence of these latter complex waveforms where the current fluctuates repeatedly between the main and the sublevel, is three orders of magnitude larger than expected by unresolved coincidence of independently occurring events. These observations are consistent with the view that the complex current waveforms represent transitions of AChR channels between a main conductance state and a substate of lower conductance.

Current-voltage relationships measured in one patch in the potential range between -50 and -120 mV (Fig. 2a) indicate that the extrapolated reversal potential for the three current levels is close to 0 mV (Fig. 2b). The substate, in this case, has a conductance of 10 pS; the conductances of the two main states are 25 pS and 35 pS, respectively.

The cation selectivity of the substate seems to be similar to that of the two main states. When Na+ in the bath solution was

Fig. 1 AChR channel currents in myoball sarcolemma. The methods used in preparing myoballs and techniques of high resolution recording and patch isolation are described in detail elsewhere 4. a. The figure on the left shows schematically the current-recording configuration used to study isolated membrane patches. Outside-out patches were formed according to the following procedure. A high pipette-membrane seal (giga-seal) obtained on the muscle cell, the initial membrane patch was disrupted by suction and the pipette then slowly withdrawn from the cell. This resulted in the formation of a new membrane patch spanning the pipette tip with its the formation of a new memorane pater spanning the pipette the with its extracellular side facing the bath solution (in mM: 150 NaCl, 1 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, 3 KCl and 10 HEPES, pH 7.2) and its cytoplasmic side facing the pipette solution (in mM: 150 KCl, 3 NaCl, 1 MgCl<sub>2</sub>, 1 EGTA and 10 HEPES, pH 7.2). The membrane potential  $V_m$  is defined as the potential of the pipette solution with respect to the bath solution. The patch current  $I_p$  is shown as a downward deflection when it flows from the bath solution into the pipette solution. Outside-out membrane patches were preferred for this study because they were typically more stable than cell-attached membrane patches, allowed ACh concentration to be adjusted during the measurements and permitted channel kinetics to be slowed by cooling to low temperatures without altering transmembrane ion gradients. The figure on the right shows the rapid activation of AChR channel currents in an outside-out patch following application (indicated by the bar) of 2  $\mu$ M ACh to the bath solution, at a potential of -70 mV, 8 °C; 400 Hz filtering. b, Oscilloscope traces showing AChR channel currents recorded at low frequency from an isolated patch of sarcolemma. The top trace is a continuous record and indicates the typical current behaviour. Two distinct classes of elementary currents  $(i_1, i_2)$  are evident. A small proportion of currents from both classes display more complex waveforms and examples are shown in the middle and lower traces. Some currents jump down to a sublevel  $(i_*)$  of ~1 pA and then either return to the resting level or jump back to the original main level. A smaller proportion of events show repetitive fluctuations between the two levels (last example in middle trace). In none of the 809 events recorded in this experiment did the sublevel precede the initial jump to the main level. In two cases the sublevel appeared in apparent isolation. Membrane potential -100 mV, temperature 8 °C. Day 14 myoball, 4 days after colchicine treatment; 1,000 Hz filtering. c, Histogram showing the relative occurrence of the three different current levels for the patch described in b. The curves are gaussian fits to the amplitudes which had mean values of 1.1, 2.9 and 4.3 pA. A total of 809 discrete current steps were recorded; 509 were in the class that had an average amplitude of 2.9 pA and 298 events were of the 4.3 pA class. Of these, 73 and 17 events, respectively, displayed the sublevel of average amplitude 1.1 pA. Three events with an average amplitude of 2.9 pA displayed a sublevel of ~2 pA, but because of the low frequency of occurrence of this level it has not been studied.



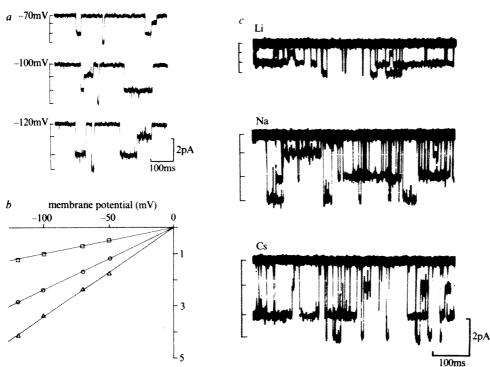


Fig. 2 Voltage dependence and ion selectivity of the three main conductance states of AChR channels. a, Current traces recorded from the same patch of myoball sarcolemma different membrane potentials. ACh concentration 1 μM, temperature 6.5 °C. Day 13 myoball, 3 days after colchicine treatment; 1.000 filtering. b, Current-voltage relationship for the three current levels measured from the patch described in a. Each data point represents the average amplitude of the level determined from 200-500 events. The lines fitted to the data points extrapolated to give reversal potential of 0 mV and yielded conductances of 10, 25 and 35 pS, c. Superimposed current traces recorded from another patch in which recordings were made in a bath solution that contained initially sodium as the pre-(150 mM), dominant cation caesium and finally lithium. Membrane potential was -100 mV. Temperature 5 °C. Day 11 myoball, 1 day after colchicine treatment; 1,000 Hz filtering. Current-voltage relationships measured in each solution over the voltage range -50 to -120 mV gave conductance values for the three levels of 4, 9 and 14 pS in Li, 8, 19 and 31 pS in Na and 11, 25 and 34 pS in Cs.

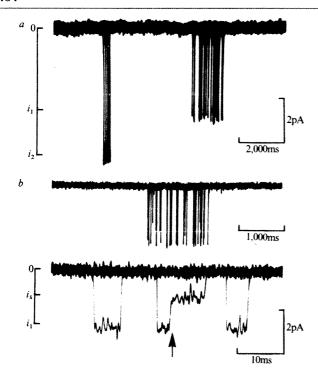


Fig. 3 AChR channel currents activated by desensitizing concentrations of ACh. a, Current recording from a sarcolemma patch activated by 10 μM ACh. Two distinct non-interacting bursts of currents were evident that corresponded in their amplitudes to the two main classes evident at low ACh concentrations. Membrane potential was -130 mV, temperature 18 °C. Day 12 myoball, 2 days after colchicine treatment; 400 Hz filtering. b, Current recording from another patch. In the top trace a burst of currents corresponding in amplitude to the smaller main level is shown at low time resolution. The bottom trace gives two superimposed traces of the same burst shown at higher time resolution. One distinct transition between the main level and a sublevel was evident and is indicated by the arrow Membrane potential -100 mV, temperature 18 °C. Day 14 myoball, 4 days after colchicine treatment; 400 and 2,000 Hz filtering.

replaced by Li<sup>+</sup> or Cs<sup>+</sup>, the conductance of all three states (Fig. 2c) decreased or increased proportionately. The various conductance states of AChR channels however differ in their average lifetime: for the patch described in Fig. 1 the average duration of the larger and smaller main levels was 12 and 36 ms, respectively, whereas the sublevel measured from the same patch had an average duration of 24 ms. However, these durations represent only apparent lifetimes because the fast fluctuations of the single channel current in the time range <1 ms (see accompanying paper<sup>5</sup>) were not taken into account. The ratio of the time the patch current is at the sublevel to the time it is at either the main or the sublevel varied between 0.02 and 0.12 in six different patches, indicating that the probability of the open channel adopting the substate is low. It is further reduced when the temperature is increased: at 18 °C current sublevels are only measurable as short notches (<5 ms) in the channel's closing time course. On the other hand, increasing the membrane potential from -70 mV to -120 mV (temperature 5 °C) increased the probability of the open channel adopting the substate from 0.05 to 0.15 (one patch). When channels have adopted the substate, the probability that they either close completely or open fully was similar. For example, in the patch illustrated in Fig. 1 the ratio of transitions back to the main state to transitions to the closed state was 1.3. Apparent transitions between the two main current levels are also observed, but their average number is not significantly different from that expected by unresolved random coincidence of two main unit current events.

These findings suggest that ACh activates two independent classes of channels in embryonic rat muscle that are distinguished by a slightly different main conductance. Both can adopt, with low probability, a similar substate of lower conductance.

This view is further supported by experiments where channels were activated by ACh at concentrations  $>2 \mu M$  where single channel currents appear in bursts. Bursts reflect sequential open-close transitions of the same AChR channel<sup>6</sup>. In embryonic muscle they fall into two main classes with respect to the size of their current pulses, corresponding to the two classes observed at low ACh concentration (Fig. 3a). Transitions between these two main current levels were never observed during a burst. However, transitions between the main level and the sublevel do occur, as shown in Fig. 3b. The upper trace shows a burst of current pulses at low time resolution, consisting of 25 well separated current events. At high time resolution two events have a complex shape characterized by a transition from the main level to the sublevel. One of these events is illustrated in the lower trace of Fig. 3b. The average probability of this channel being in the substate (given the channel is in the open state), as determined from eight consecutive bursts, was 0.08. This experiment shows directly that a single AChR channel complex, in the presence of constant ACh concentrations, fluctuates between at least three states: a main open state, a substate which is adopted at low probability, and the closed

The AChR complex purified from Torpedo electroplaque is a pentameric protein complex comprised of five homologous subunits<sup>7</sup> which span the membrane<sup>8</sup>. In lipid bilayers it can form a unit conductance channel which has a similar conductance to that of the AChR channel in rat muscle9. The observation that ACh-activated channels in embryonic muscle cells can adopt several conductance states could indicate that these subunits rearrange themselves to form the different open states of a channel. Similarly the two independent classes of channels may represent two different aggregation states of the same set of subunits.

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#### Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels

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Acetylcholine-like drugs cause ion channels in the skeletal muscle endplate to open briefly1, producing, at random intervals, rectangular pulses of current with constant amplitude but random duration, that can be recorded by the patch clamp method<sup>2,3</sup>. However, even when the agonist concentration is so low that channel activations are very well separated, we have observed, with high resolution methods4, that openings may be interrupted by shut periods (gaps) so brief that they are very unlikely to arise from two independent channel activations. This sort of behaviour has been predicted on the basis that two or more openings might occur during the time for which the receptor remains occupied by agonist<sup>5,6</sup>. If this were correct, important new information about agonist activation of ion channels could be obtained from measurements of the gaps between openings. However, short gaps could arise in other ways: for example from brief blockage of the ion channel<sup>7</sup>, perhaps by the agonist itself. We now present results obtained with the acetylcholine-like agonist, suberyldicholine (SubCh, 20–100 nM), which suggest that the brief gaps do not result from ion channel block by the agonist itself, but which are consistent with a mechanism in which the channel opens and closes several times during a single agonist receptor occupancy. We have also observed that the number of short (<1 ms) current pulses is greater than we expected.

Single ion channel currents from the perisynaptic region of normal frog (*Rana temporaria*) cutaneus pectoris muscle were recorded at 10–13 °C (ref. 4). The Ringer solution contained (mM) NaCl 115, KCl 2.5, CaCl<sub>2</sub> 1.8 and HEPES buffer 3, pH 7.2

Figure 1a shows the typically low frequency of single channel currents (always  $<3\,\mathrm{s}^{-1}$ ). Individual currents are shown, with high time resolution, in Fig. 1b,c; they are clearly interrupted by brief gaps. During most of these gaps the current does not reach the resting value; however, the frequency response of the system is such that the channel would have to be shut for nearly  $300\,\mu\mathrm{s}$  for this to be attained. We assume that the gaps represent brief, but complete, closures of the ion channel, and Fig. 1d–g show how the duration of such closures was estimated. In good records, events with a duration of 50– $70\,\mu\mathrm{s}$  could be clearly resolved.

The entire record was digitized so that the duration of all resolvable gaps, as well as openings, could be measured. After this measurement, a safe value was chosen for the minimum resolvable duration, and the record was revised by concatenation of adjacent gaps and openings separated by intervals less

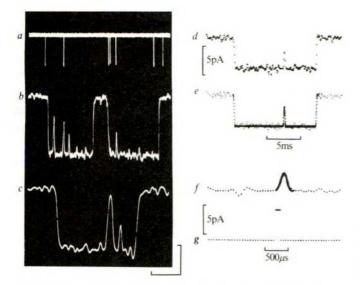
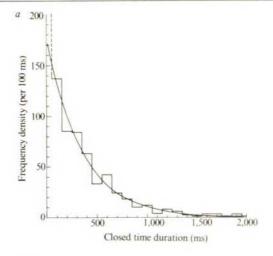


Fig. 1 Examples of single channel currents (a, b, c) and the method used to fit their time course (d, e, f). Low pass filter set at 4 kHz. a, Illustration of low frequency of events (seven in 5 s in this case) with 100 nM SubCh at -123 mV. Calibration bars: 4 pA and 1 s. b, Several brief closures interrupting an open channel current; taken from the experiment illustrated in a. Calibration bars: 2 pA and 10 ms. c, Another group of three openings, at higher time resolution than in b; 20 nM SubCh at -128 mV. Calibration bars: 2 pA and 2 ms. d, A burst with one resolvable gap, digitized at 16 kHz; 50 nM SubCh at -171 mV. e, The same data as in d with a fitted line (heavy line) superimposed on it. The response of the recording system to a step input was measured experimentally, and four such response functions, with alternating direction, were convolved to generate the fitted line. f, The gap shown in d and e, with greatly expanded time scale, but the same amplitude scale. A fitted line is superimposed on the data to illustrate the fitting of brief gaps. This fitted line was generated, as explained above, as the expected response of the system to the input shown in g. This fit estimates that the burst shown in d consists of two openings of length 7.69 and 4.58 ms, separated by a gap of 104 µs, so the total length of the burst is 12.37 ms.



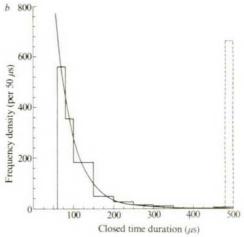
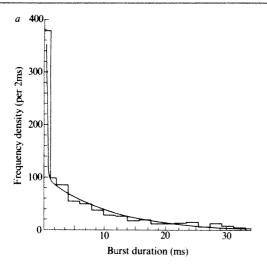


Fig. 2 Distribution of the durations of all closed times (gaps) between open channel currents. Experiment with 20 nM SubCh at -131 mV. Ordinate is frequency density (number of observations per time interval of the specified length). All 1,308 gaps that were measured were simultaneously fitted (as described in the text) with the sum of three exponentials, and the fitted curve was superimposed on the histogram of the observations which is shown with two different time scales in a and b. In this case we found components with time constants of 352 ms (representing 20.5% of total area under the distribution), 45 μs (76.7% of area) and 0.66 ms (2.8% of area). a, Distribution of gaps, shown up to 2,000 ms, with fitted curve. The leftmost bin (dashed line) contains many short gaps, and extends well off the graph. b, The same distribution, shown up to 500 μs, only, with fitted curve. The resolution was fixed (see text) at 60 μs in this experiment. The rightmost bin (dashed line) represents all gaps longer than 500 μs. This includes all those shown in a.

than this chosen resolution. Thus an idealized record, with consistent resolution throughout, was obtained for the construction of histograms. All distributions of time intervals were fitted with the sum of one or more exponential functions by the method of maximum likelihood (that is, the actual measured durations were used, not the histogram frequencies).

The distribution of all gaps is exemplified in Fig. 2. In Fig. 2a, the time scale extends up to 2 s, and the gap durations are fitted well by an exponential component with a mean of 352 ms. This is presumably the mean interval between independent activations of ion channels. However, the first bin (up to 50 ms) extends a long way off the graph; the distribution of short gaps is shown in more detail in Fig. 2b, in which the time scale extends up to only  $500 \, \mu s$ . There is a clear exponential component of the gap distribution with a mean, in this example, of  $45 \, \mu s$ , which corresponds to the short gaps illustrated in Fig. 1. Values of  $45-70 \, \mu s$  were consistently observed in nine other experiments. This fast component represented, in this case, 77 per cent of the total area of the gap duration distribution, corresponding to a total number of 2,231 short gaps (although many of these would



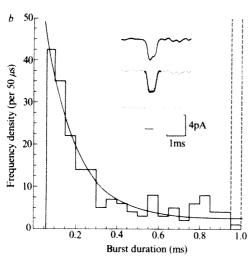


Fig. 3 Distribution of the burst duration, as defined in the text, for the same experiment as shown in Fig. 2. With a resolution of 60 µs and critical gap length of 1.5 ms, there were 683 bursts altogether. The durations of these bursts were fitted (see text) with the sum of two exponentials. In this case we found components with time constants of 10.2 ms, which is similar to the value expected from a noise analysis in the conditions of this experiment, and 0.15 ms. In 12 experiments the average faster time constant was  $0.35 \pm$ 0.12 ms. a, The distribution of burst length, and fitted curve, shown up to 35 ms. b. The same distribution and fitted curve, but bursts up to 1 ms duration only are shown. The rightmost bin (dashed line) represents all bursts longer than 1 ms. The inset shows (top) an example of a brief opening. This is fitted (see Fig. 1, right column) with a superimposed line (middle) calculated as the response to the step input shown (bottom). The amplitude (3.95 pA) was taken as the average of amplitudes of all full size openings previously fitted in this experiment. The duration is 495 µs.

be too short to resolve). In addition we have consistently found a much smaller component (about 2% of total area) with an intermediate time constant of the order of 1 ms, but this will not be considered further here.

The evidence presented above suggests that the 'openings' will contain many unresolved gaps, and are thus not well defined. We therefore define a burst of openings as any series of 'openings' interrupted by gaps that are all less than some critical length (usually 0.5-3 ms). Insofar as this length is very much smaller than the mean time between independent events (352 ms in Fig. 2), the burst duration is a well defined quantity. This occurrence of openings in quick succession was called the Nachschlag phenomenon when it was first observed, and this term seems appropriate, whatever the mechanism responsible for the phenomenon. Figure 3 shows the distribution of burst durations; in Fig. 3a the time scale extends to 35 ms, and there are obviously too many short bursts to be fitted by a single exponential distribution. The double exponential distribution shown has a slow component with mean of 10.2 ms, and a fast component with mean of 0.15 ms. The total area under the distribution represents 717 bursts and the slower component corresponds to 73% of the total area in this example, with 20 nM SubCh. Thus we infer, for the experiment illustrated in Figs 2 and 3, that there are, on average 2,231/717 = 3.1 gaps per burst. Similar values (2-4) were found in 10 experiments.

The origin of the short openings illustrated in Fig. 3 is obscure. They may represent a separate type of channel altogether, or perhaps brief openings by channels with only one agonist molecule bound. We shall explore these hypotheses elsewhere.

The most important question that arises is whether the brief gaps result from ion channel block by the agonist, SubCh, itself. Decamethonium (in much higher concentrations) is known to do this<sup>8</sup>. If this were the mechanism, the number of gaps (blockages in this case) per burst should be directly proportional to the agonist concentration. However, the number of gaps per burst with 100 nM SubCh, relative to that with 20 nM, was  $0.97 \pm 0.11$ (three experiments at each concentration), and showed little sensitivity to membrane potential. Therefore, ion channel block by SubCh, in the low concentrations used, cannot explain the brief gaps that we observe. Although it is conceivable that brief closures of the channel might arise from block by some endogenous muscle constituent, or from a mechanism connected with ion permeation, the most plausible alternative to ion channel block is that the closures arise from multiple openings during a single receptor occupancy<sup>5,6</sup>. Suppose, for example, that two agonist molecules must bind sequentially before the channel can open (see, for example, ref. 9). Then (see ref. 6), at low agonist concentration, the mean length of a gap should be approximately  $(\beta + 2k_{-2})^{-1}$ , and the number of gaps per burst should be approximately  $\beta/2k_{-2}$ , where  $\beta$  is the rate constant for opening of a doubly occupied receptor-channel complex, and  $k_{-2}$  is the microscopic rate constant for dissociation of an agonist molecule. Our results, if interpreted in this way, thus suggest preliminary estimates for  $\beta$  of the order of  $10,000-15,000 \text{ s}^{-1}$ , with  $k_{-2}$  roughly  $2,000 \text{ s}^{-1}$ . This interpretation of our results with SubCh is not compatible with the common assumption that the agonist binding step is very fast, and that the open-shut conformation change is rate-limiting<sup>10</sup>. We are now investigating whether or not the same can be said of agonists other than SubCh. The noise spectrum expected if this interpretation were correct would consist predominantly of a single component, with a time constant close to the mean length of the burst (slow component), that is, 10 ms in the experiment shown in Fig. 3. But this time constant would not correspond, as is often assumed, to the true lifetime of the open state. In the above example the average burst consists of roughly four openings in quick succession, so the mean open lifetime,  $1/\alpha$  (where  $\alpha$  is the channel closing rate constant) would be only about 2.5 ms.

Results similar, in some respects, to ours have recently been found (S. G. Cull-Candy and I. Parker, in preparation) for glutamate-operated channels in locust muscle.

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#### A specific substance P antagonist blocks smooth muscle contractions induced by non-cholinergic, non-adrenergic nerve stimulation

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Nerve fibres containing substance P (SP) are widely distributed in the body1-3 and seem to innervate autonomic ganglia, blood vessels, epithelial structures and smooth muscle. SP stimulates secretion from exocrine glands, causes vasodilation and contracts non-vascular smooth muscle4. The presence of SP in primary sensory neurones has lent support to the view that it is associated with sensory nerve conduction, conceivably as a transmitter5, and that it is a causative factor in the 'irritative' response to antidromic stimulation of sensory nerves°. Gut smooth muscle contracts in response to non-cholinergic, non-adrenergic nervous stimulation<sup>7,8</sup>, and it has been suggested that SP acts as an excitatory transmitter in intramural neurones in the gut wall<sup>2,3,9,10</sup>. Recently, a series of synthetic analogues of SP with antagonist activity to SP has been developed 11,12. We report here that a new analogue, (D-Pro2, D-Trp7.9)-SP, exercises a specific, possibly competitive antagonism to SP. While being a partial agonist it antagonized the contractile response to applied SP and to non-cholinergic, non-adrenergic nerve stimulation in the isolated guinea pig taenia coli and rabbit iris sphincter pupillae muscle, suggesting that SP, or a closely related peptide, is indeed a motor excitatory transmitter. In contrast, (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP did not inhibit the contractile response to non-cholinergic, non-adrenergic nerve stimulation of smooth muscle from the guinea pig urinary bladder.

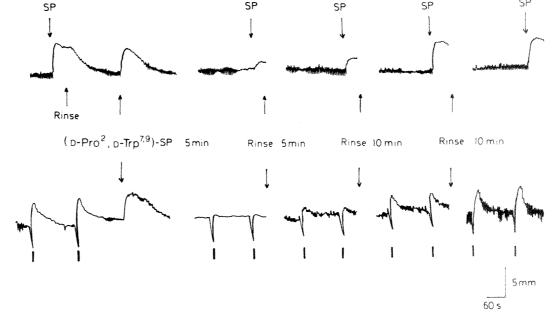
Guinea pig taenia coli preparations, consisting of longitudinal smooth muscle with the attached myenteric plexus<sup>13</sup>, were

placed in Krebs solution, stored at 4°C for ~1 h and then mounted vertically on a Perspex holder in a 7-ml organ bath thermostated at 37 °C. One end was attached to a rigid support and the other to a lever connected via a spring to a Grass FT03 force displacement transducer or to a photoelectric transducer for isotonic registration of mechanical activity. The load on the muscle was set at 0.2 g. Platinum ring electrodes were placed around the muscle with a constant electrode distance of 5 mm and the electrodes were connected to a Grass S 4C stimulator for field stimulation with square wave pulses (15 V over the electrodes, 0.5-1 ms duration). The muscles were stimulated with trains of pulses lasting 3 s and with a frequency of 1-5 Hz, the resting period between stimulations being at least 2 min. The mechanical activity of the preparation was continuously recorded on a Grass model 7 or model 5 polygraph. The bathing fluid was a modified Krebs solution of the following composition (in mM): NaCl 133, NaHCO $_3$  16.3, KCl 4.7, MgCl $_2$  1.0 NaH $_2$ PO $_4$  1.4, CaCl $_2$  2.5 and glucose 7.8. The solution was bubbled with a gas mixture of 7% CO2 in O2 giving a pH of 7.2 - 7.3.

The sphincter pupillae muscle of the rabbit iris was excised, opened and mounted as described for the taenia. Mucosa-free strips of the detrusor of the guinea pig bladder were prepared as described in detail elsewhere 14. The same procedure as above was used to study the motor activity of the sphincter pupillae and the urinary bladder except that they were placed directly in the bath, experiments with sphincter pupillae muscle were run at 35 °C, and the stimulation parameters were 10–20 Hz for 10 s for the sphincter pupillae muscle 15 and a single pulse every 20 s for the urinary bladder. (D-Pro², D-Trp².9)-SP was synthesized by techniques analogous to those described elsewhere 11.

The addition of SP and of (D-Pro<sup>2</sup>, D-Trp<sup>7.9</sup>)-SP to guinea pig taenia coli produced a contraction which lasted  $120-150 \,\mathrm{s}$ , the tension subsequently returning to baseline (Fig. 1). As illustrated in Fig. 2a the contractile effect of the SP analogue required high concentrations. The contractile response to exogenous SP was strongly inhibited by pretreatment with (D-Pro<sup>2</sup>, D-Trp<sup>7.9</sup>)-SP (Fig. 1), the blockade lasting for at least 30 min without washing. Washing out the SP analogue resulted in a return of the contractile response to exogenous SP and to electrical stimulation (Fig. 1). The analogue also reduced the contractile response to exogenously applied physalaemin and eledoisin but not that to carbachol, histamine, serotonin, bradykinin, Lys<sup>8</sup>-vasopressin or prostaglandin  $F_{2\alpha}$  (Fig. 3). In the presence of (D-Pro<sup>2</sup>, D-Trp<sup>7.9</sup>)-SP, the dose response curve

**Fig. 1** Effect of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP  $(10^{-5} \text{ M})$  on the contractile response to exogenous substance P (SP; Peninsula) (5×10<sup>-9</sup> M) Peninsula) (upper panel) and to the electrically evoked contraction (lower panel) of isolated guinea pig taenia coli. The stimulated in the presence of atropine ( $10^{-6}$  M; ACO) and guanethidine ( $5 \times 10^{-6}$  M; Sigma) for 3s with 3 Hz as indicated by black rectangles. Note the agonist effect of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP, the ensuing blockade of the contractile response to exogenous SP and the reduction of the electrically induced contraction on addition of the SP analogue. Electrical stimulation applied 5 min after contractile effect of (D-Pro2, D-Trp7.9)-SP had subsided.



The inhibitory effect of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP on smooth muscle contractions can be washed out as illustrated by the recovery of the contractile response of the taenia coli to exogenous SP and to electrical nerve stimulation. Rinsings and time intervals between registrations are indicated. The registrations shown are typical examples of four to six experiments,

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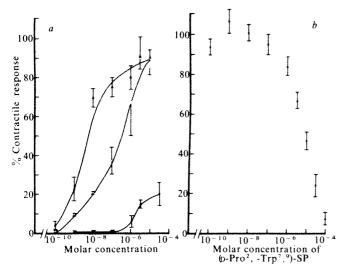


Fig. 2 a, Dose-response curves showing the effects of SP alone ( $\triangle$ ), of SP in the presence of (D-Pro2, D-Trp7.9)-SP (O), and of (D-Pro2, D-Trp7.9)-SP alone (●) on the tension of the guinea pig taenia coli, expressed as percentage of the contraction induced by carbachol (10<sup>-5</sup>; Merck-Darmstadt). In the presence of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP (10<sup>-5</sup> M) the dose-response curve for was shifted 2.54 log units to the right, suggesting competitive inhibition. The dose-response curves were constructed by adding step wise increasing amounts of peptides to the bath. Below  $10^{-7}$  M three concentrations of SP were tested on each muscle; above  $10^{-7}$  M only one concentration was tested per muscle. The corresponding concentration for the SP analogue was 10<sup>-6</sup> M. SP was added in a volume of 200 μl, the SP analogue in 40 μl. Between each addition the bath was rinsed until the tension had returned to base line (usually three rinses, in all 5-10 min). Each value is the mean of 3-10 experiments. Vertical bars give s.e.m. b, Effect of increasing concentrations of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP on the contractile response of the isolated guinea pig taenia coli to electrical stimulation (3–5 Hz, 3 s). Atropine  $(10^{-6} \, \mathrm{M})$  and guanethidine  $(5 \times 10^{-6} \, \mathrm{M})$  were present in the bath. New muscles were set up for each concentration tested. Electrical stimulation was applied 5 min after the contractile response to the SP analogue had subsided. The results are expressed as per cent of the contractile responses before adding the SP analogue. Each value is the mean of 4-18 experiments. Vertical bars give s.e.m.

for SP was shifted to the right in a manner suggesting competitive inhibition (Fig. 2a). Desensitization with SP is a well known phenomenon, but it can be ruled out as an explanation for the inhibitory effect of the SP analogue because high concentrations of SP ( $>10^{-6}$  M) could overcome the inhibition, very high concentrations ( $>10^{-8}$  M) giving a maximal contractile response.

Électrical stimulation (1-5 Hz, 3 s) of the taenia elicited a strong contraction, and this response was not affected by addition of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP. Following addition of the muscarinic blocker atropine (10<sup>-6</sup> M) and the anti-adrenergic drug guanethidine (5 × 10<sup>-6</sup> M), the taenia responded to low-frequency stimulation (1 Hz, 3 s) by relaxing. With higher-frequency stimulation (3-5 Hz, 3 s), the initial relaxation was followed by a contraction on cessation of stimulation<sup>10</sup>. Both these responses were inhibited by tetrodotoxin, indicating a neuronal mediation<sup>16</sup>. Although relaxation was unaffected, the amplitude of the contraction was greatly reduced by pretreatment with (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP (Fig. 1). Interestingly, the dose-response curve revealed that a 10<sup>-4</sup> M concentration of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP reduced the contraction by more than 90% (Fig. 2b).

The iris sphincter pupillae muscle responded to both SP and electrical stimulation with a slow contraction, in the latter case reaching a maximum 30-40 s after cessation of stimulation. The electrically induced contraction lasted for  $\sim 150$  s. Cholinergic and adrenergic blockade had very little effect on the response to electrical stimulation, but (D-Pro², D-Trp², )-SP caused a dramatic reduction in the contractile response (Fig. 4a). The remaining contraction was further reduced by adding tetrodo-

toxin. As in the taenia coli, the response of the iris sphincter muscle to exogenous SP, physalaemin and eledoisin (but not that to carbachol) was greatly inhibited by pretreatment with (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP.

The guinea pig urinary bladder responded to both SP and electrical stimulation with a contraction. Cholinergic and adrenergic blockade had little or no effect on the response to electrical stimulation<sup>14</sup>. Also, (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP failed to inhibit the response, which could be completely abolished by tetrodotoxin (Fig. 4b).

The results of these experiments indicate that (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP is a partial agonist, exercising a specific, possibly competitive antagonism to SP. Interestingly, (D-Pro<sup>2</sup>, D-Trp<sup>7</sup>, also blocked the effects of physalaemin and eledoisin. Together these peptides have been grouped under the name tachykinins<sup>17,18</sup>; they have the carboxy-terminal three amino acids in common and display smooth muscle stimulating effects<sup>17,18</sup>. Following cholinergic and adrenergic blockade, electrical nerve stimulation still induced a contraction of the guinea pig taenia coli, urinary bladder and rabbit sphincter pupillae muscle. In the taenia coli and sphincter pupillae muscle these contractions were greatly reduced by (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP, suggesting that SP is involved as a motor excitatory transmitter. However, the SP analogue did not inhibit the contractile response to electrical nerve stimulation of the urinary bladder following cholinergic and adrenergic blockade. Hence, SP is not in all tissues the only non-cholinergic, non-adrenergic motor excitatory neurotransmitter. SP nerve fibres in the smooth muscle of the taenia coli probably originate from cell bodies in the mysenteric plexus 10,19-21, but their precise nature and mode of action remain to be established. Our results suggest that they are engaged in an efferent motor excitatory capacity although it cannot be excluded that SP is released as a result of antidromic nerve stimulation. The SP fibres in the sphincter pupillae muscle

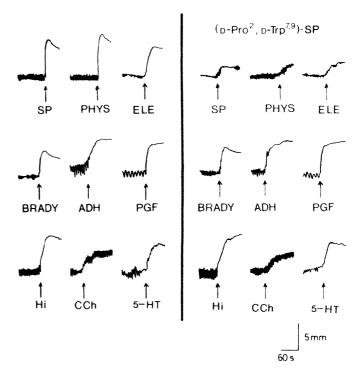
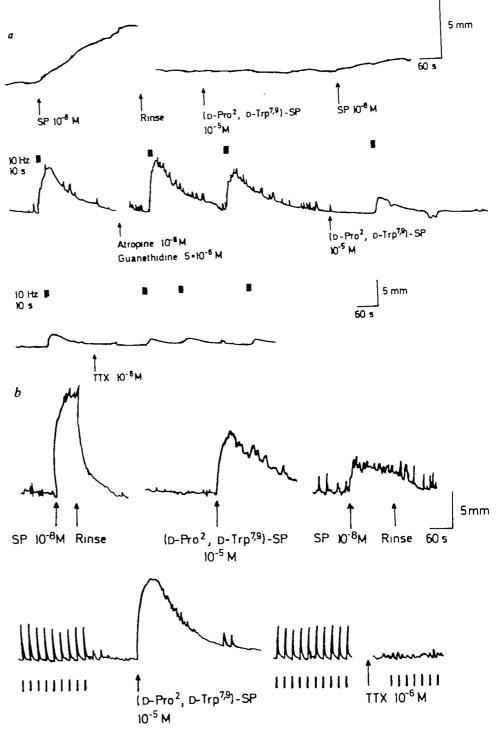


Fig. 3 Contractile response of the isolated guinea pig taenia coli to physalaemin (PHYS), eledoisin (ELE; both Peninsula) at a concentration of  $10^{-9}\,\rm M$ , SP bradykinin (BRADY; Beckman), histamine (Hi; Merck-Darmstadt), carbachol (CCh) and serotonin (5-HT; Sigma) at a concentration of  $10^{-8}\,\rm M$ , Lys\*-vasopressin (antidiuretic hormone, ADH; Ferring) and prostaglandin  $\rm F_{2\alpha}$  (PGF; Sigma) at a concentration of  $10^{-7}\,\rm M$ , with (right) or without (left)  $10^{-5}\,\rm M$  (D-Pro², D-Trp²-9)-SP in the bath. The contractile effect of the SP analogue was allowed to subside before the other compounds were added, usually 5 min later.

Fig. 4 a, Effect of (D-Pro2, D-Trp7.6)-SP on the contractile response to exogenous SP (upper panel) and to the electrically evoked contraction (middle and lower panels) of the rabbit iris sphincter pupillac muscle. In this preparation the SP analogue per se had no contractile effect. Electrically induced contraction per sisted in the presence of atropine and guanethidine. Note the absence of contractile effect of exogenous SP and the reduction of the electrically evoked contraction following addition of the SP analogue, Addition of tetrodotoxin (TTX. Sankyo) almost completely abolished the residual contractile response b, Effect of (p-Pro<sup>2</sup>, p-Trp<sup>7,9</sup>)-SP on the contractile response to exogenous SP (upper panel) and to the electrically evoked contraction (lower panel) of the gumes pig urinary bladder. The muscles were electrically simulated in the presence of atropine  $(10^{-6} \text{ M})$  and guanethidine  $(5 \times 10^{-6} \text{ M})$ with a single pulse every 20 s as indicated Note the agonist effect of (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP, the ensuing blockade of the contractile response to exogenous SP and the lack of effect on the electrically induced contraction on addition of the SP analogue, Electrical stimulation or exogenous SP were applied 5-10 mm after the contractile effect of (D-Pro<sup>2</sup>, D-Trp<sup>2</sup>)-SP had subsided. Addition of TTX completely abolished the contractile response to the electrical field stimulation. The results shown in a and b are typical examples from a series of four to six experiments



are probably derived from the trigeminal nerve 15,22,23, which is assumed to have a sensory function. Hence, the SP-mediated contraction is either the result of antidromic nerve stimulation or an indication that the trigeminal nerve carries efferent fibres.

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# Distinct subpopulations of enteric p-type neurones contain substance P and vasoactive intestinal polypeptide

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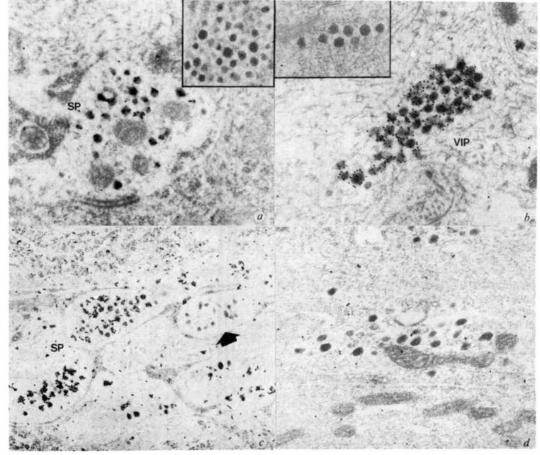
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Enteric nerve profiles ultrastructurally distinct from the adrenergic and cholinergic types have been recognized for more than a decade1. Baumgarten and his colleagues2 called these neurones p-type (peptidergic) because they contain large, granular, secretory vesicles similar to those found in peptidecontaining nerves in the brain. Subsequently ultrastructural heterogeneity within this extensive non-adrenergic, non-cholinergic p-type system was described, based on the appearance of these granular vesicles3. This was consistent with the light microscopical demonstration of increasing numbers of peptides in the gut innervation<sup>4,5</sup>, and has been further supported by the localization of vasoactive intestinal polypeptide (VIP) immunoreactivity within large p-type secretory vesicles in the cat proximal colon6. Using electron-immunocytochemical techniques on aldehyde-fixed tissue we have shown that the morphological heterogeneity of the p-type system is partly due to the storage of different peptides. We present here evidence that two peptides, VIP and substance P (SP), are present in separate subpopulations of p-type neurones distinguishable by the size and appearance of their granular secretory vesicles. Further subpopulations, unlabelled by specific anti-SP and anti-VIP sera, are also present.

A colloidal gold-labelled second layer antibody was used in a two-step immunocytochemical technique based on the original method of Romano and his colleagues7. Purified goat IgG was labelled with colloidal gold particles measuring 20 nm in diameter<sup>8</sup> (see also Fig. 2 legend), in a technique modified from existing methods<sup>7,9-11</sup>. Antisera to SP and VIP were raised in . Antisera to SP and VIP were raised in rabbits and characterized for immunocytochemistry (Table 1 and Fig. 2 legend). The colon from adult guinea pigs (n = 15) was used (Fig. 1 legend). The method for immunostaining ultrathin sections was similar to that described by Roth et al. 12, Larsson 1 and Batten and Hopkins<sup>14</sup>. Briefly, ultrathin sections were etched with 10% H<sub>2</sub>O<sub>2</sub> for 15 min, incubated with normal goat serum (1/30 dilution) and then either SP or VIP antiserum for 18 h at 4 °C (Table 1). The sections were rinsed with 0.05 M Tris-buffered saline pH 7.3 (TBS) containing 0.2% bovine serum albumin (BSA)7, before incubation with gold-labelled goat anti-rabbit IgG for 1 h at room temperature (see Fig. 2 legend). Following another wash in TBS/BSA buffer the sections were counterstained with uranyl acetate and lead citrate. Controls were carried out as described in Fig. 2 legend.

Throughout the wall of the guinea pig colon, SP- and VIP-like immunoreactivities were consistently found in the large granular secretory vesicles present in separate and distinct sub-populations of p-type neurones (Fig. 1). Furthermore, these neurones could be distinguished by the size and appearance of the secretory vesicles. Immunoreactivity was observed mainly in

Fig. 1 Ultrathin sections of guinea pig myenteric plexus showing nerve terminals: a, immunostained with antiserum (SP) (resin polymerized at 60°C); ×30,000 (inset showing control section stained using SP antiserum preabsorbed with SP antigen (see Table 1); ×30,000). Immunostained with antiserum (VIP); ×30,000 (inset showing control section stained with VIP antiserum preabsorbed with VIP antigen (see Table 1); ×30,000. Immunostained with antiserum showing positive (SP) and negative terminals (resin polymerized 60°C); ×20,000. Immunostained with antiserum (also negative following application of SP antiserum); ×30,000. Note the specific gold labelling over the large, granular, p-type vesicles present in the nerve terminals shown in a and b. Control sections, as shown in the insets, were completely unstained. The colon was removed from adult Dunkin Hartley guinea pigs under pentobarbitone anaesthesia. Proximal, middle and distal regions were diced and immersion fixed in a cold solution of 3% glutaralde-hyde in 0.1 M phosphate buffer pH 7.3, for 3 h at 4°C. The tissue was washed over-



night in 0.1 M phosphate buffer pH 7.3 containing 0.1 M sucrose, dehydrated and embedded in Araldite. Resin was polymerized either at 60 °C (SP) or at 18 °C using UV light (SP and VIP). Ultrathin sections were cut on a Reichert Ultracut microtome, collected on uncoated 300-mesh nickel grids and immunostained as described in the text. In addition, alternate serial sections from blocks polymerized using UV light were stained with either SP or VIP antiserum so that a direct comparison of vesicle types could be made. A statistical comparison of the results was carried out using an unpaired Student's r-test.

nerve terminals and varicosities although VIP-immunoreactive vesicles were occasionally seen in cell bodies of the submucous plexus (Fig. 2). SP-immunoreactive neurones are also known to be present in the guinea pig gut<sup>4,15</sup> but none was identified in this study. As an extensive study of samples throughout the colon of each animal (n = 15) was undertaken, this may be explained either by the presence of a non-reactive precursor form, or a concentration too small to be detected by immunocytochemistry in the cell bodies. Optimal immunostaining of SP and VIP was obtained following different resin polymerization schedules (see Fig. 1 legend). To achieve an accurate comparison of SP- and VIP-immunoreactive vesicles, staining for both peptides was carried out in parallel on alternate serial sections from blocks polymerized at 18 °C with UV light. Section thickness and staining density were standardized as much as possible as these factors are known to affect the electron density of ultrathin sections. Interestingly, the different methods of resin polymerization produced no difference in the size of the vesicles, although they did appear to be slightly less electron dense in the blocks polymerized at the lower temperature. Both SP- and VIP-immunoreactive vesicles were predominantly spherical in shape, although occasionally ovoid or slightly elongated immunoreactive vesicles were observed in terminals of each type. Comparison of the serial sections taken from low temperature cured blocks showed the VIP-immunoreactive vesicles to be significantly larger (mean ± s.d.) (98 ± 19 nm diameter) than the SP-immunoreactive vesicles ( $85 \pm 15 \text{ nm}$ diameter) (P < 0.01, see Fig. 1 legend), and more electron dense. At least two further subclasses of p-type nerve terminals were present which remained unstained by SP or VIP antiserum. These contained (1) mainly elongated vesicles of medium electron density measuring  $74 \pm 11 \times 91 \pm 25$  nm (Fig. 1c), and (2) large (105±31 nm), spherical vesicles with a homogeneous, dense core (Fig. 1d).

We present here evidence that within the heterogeneous p-type innervation of the guinea pig gut, two of the most abundant gut neuropeptides, SP and VIP, are stored in distinct types of nerve terminals distinguishable by the size and appearance of their large, dense-cored secretory vesicles. In addition, further subclasses of p-type terminals, unstained by either SP or VIP, can be seen. The present stage of the classification of peptide-containing nerves is reminiscent of the early description of all gut endocrine cells as enterochromaffin cells16. These were subsequently subdivided ultrastructurally17, but it was not until immunocytochemical techniques were applied that a functional classification could be attempted. This led to the identification of the peptides present in 14 out of the 18 cell types 18. The original p-type terminals have reached the stage of morphological subclassification3 and two subtypes have been reclassified by immunocytochemistry. Of the nine types of nerve profiles described, three variations of p-type terminal were reported by Cook and Burnstock3 according to vesicle size and appearance. The types 5b (see their Fig. 28) and 5c (see their Fig. 29) resembled most clearly the SP- and VIP-immunoreactive vesicles, respectively, as described here. Conclusive identification is difficult because fixation conditions in the two studies were not identical. As a wide range of biologically active peptides is known to be present in the innervation of the mammalian gut4.5, it is likely that at least some of these will be localized to further kinds of p-type terminal. The availability of

Table 1 Characterization of antisera for immunocytochemistry

Absorption (1–10 nmol per Antiserum ml diluted antiserum)								
to	Reference	Titre	BN	VIP	SP	GIP	GLUC	SEC
VIP	(652)	1/6,000	+	-(5)	+	+	+	+
SP	(479)	1/8,000	+	+	-(3)	+	+	+

Reference: antiserum reference number. Controls included the application of antiserum preabsorbed with bombesin (BN), gastric inhibitory polypeptide (GIP), glucagon (Gluc), secretin (Sec), VIP and SP; +, no change in the intensity of immunostaining; -(nmol ml-1), minimum amount of peptide required to abolish the immunostaining.

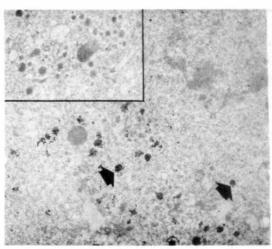


Fig. 2 Ultrathin section of guinea pig submucous plexus showing a VIPimmunoreactive neuronal cell body. Note the specific gold labelling over the large p-type vesicles present in the cytoplasm (arrows); ×20,000 (inset showing serial section stained with VIP antiserum preabsorbed with VIP antigen (see Table 1); ×20,000). SP and VIP antisera were raised in rabbits using synthetic SP (Beckman) coupled to thyroglobulin, and natural porcine VIP (donated by Professor V. Mutt, Karolinska Institute, Stockholm) coupled to haemocyanin with glutaraldehyde, respectively. Both antisera were characterized for immunocytochemistry and found to be directed to the C-terminal portions of their respective molecules. Controls for immunocytochemistry included incubation with the following in place of the first layer antiserum: (1) SP or VIP antiserum preabsorbed with synthetic SP or VIP antigen respectively (1-10 nmol ml<sup>-1</sup> diluted antiserum, see Table 1); (2) non-immune serum; (3) gold-labelled antibody alone; (4) SP or VIP antiserum preabsorbed with other gut peptides (up to 40 nmol ml-1 diluted antiserum). Control sections treated as in (1) to (3) were devoid of specific gold labelling. In control (4) labelling for either SP or VIP was not diminished by the addition of any other gut peptide including bombesin, a peptide known to share two C-terminal amino acids with SP, and glucagon, secretin or gastric inhibitory polypeptide (GIP), which are structurally related to VIP (see Table 1). Gold-labelled goat IgG was prepared8. Briefly, purified goat anti-rabbit IgG was dialysed against 2 mM borax buffer pH 9.0. Protein aggregates were removed by centrifugation (100,000g for 1 h at 4 °C). The optimal amount of antisera necessary to stabilize the gold solution10 was added dropwise with stirring to the gold solution at room temperature. After 1–2 min stirring a stock solution of bovine serum albumin (BSA) (10% in water) was added to a final concentration of 1%. Unbound proteins were removed by three cycles of centrifugation (14,000g for 1 h at 4 °C in 1% BSA/TBS buffer pH 8.2 + 2 × 10<sup>-2</sup> M sodium azide). The last pellet was resuspended to give a final volume 1/10 that of the original gold solution. For immunocytochemistry a dilution of 1/4 was made in TBS containing 0.2% BSA.

immunocytochemical methods for use on ultrathin sections, such as those using colloidal gold<sup>8,12-14</sup>, which allow the visualization of a specific immunoreaction with minimal loss of ultrastructural detail, will be invaluable in establishing the full heterogeneity of the gut p-type innervation.

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# GABA reduces binding of ${}^{3}$ H-methyl $\beta$ -carboline-3-carboxylate to brain benzodiazepine receptors

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Benzodiazepines are thought to exert their pharmacological and clinical effects by interacting with specific receptors on neurones in the central nervous system<sup>1-3</sup>. Originally, only benzodiazepinoid compounds were known to interact with these receptors, but recently other classes of agents have been discovered which have high affinity for benzodiazepine receptors4. A representative from one of these classes, ethyl  $\beta$ -carboline-3carboxylate ( $\beta$ -CCE), was obtained from human urine by virtue of its high affinity for benzodiazepine receptors. It was hypothesized that derivatives or congeners of this  $\beta$ -carboline could be related to presumed endogenous ligands, the exact nature of which are unknown<sup>3,5</sup>. Another ester of β-carboline-3-carboxylic acid, the propyl ester, PrCC, has recently been used as a radioligand for labelling benzodiazepine receptors6: in particular, <sup>3</sup>H-PrCC has been observed selectively to label a BZ<sub>1</sub> receptor subclass7. Binding of PrCC to benzodiazepine receptors, however, was less enhanced by  $\gamma$ -aminobutyric acid (GABA) than expected<sup>6,8</sup>. The affinity of benzodiazepines for benzodiazepine receptors is enhanced two to threefold by GABA<sup>9-11</sup>, probably reflecting the functional coupling of benzodiazepine receptors and GABA receptors at the molecular level. Here we have investigated binding of the methyl ester of  $\beta$ -carboline-3-carboxylic acid ( $\beta$ -CCM), which by itself is a convulsant, in contrast to  $\beta$ -CCE and PrCC. We report that <sup>3</sup>H-β-CCM binds to brain benzodiazepine receptors and that, in contrast to binding of <sup>3</sup>H-diazepam, <sup>3</sup>H-β-CCM binding is reduced by GABA in a bicuculline-sensitive manner.

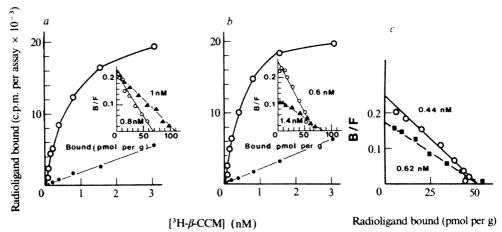
<sup>3</sup>H-β-CCM bound readily to membranes prepared from rat forebrain, hippocampus and cerebellum. Equilibrium was achieved in 10 min at 0 °C and did not change for at least 80 min (data not shown). Specific binding of <sup>3</sup>H-β-CCM was defined as

the difference between total binding and nonspecific binding, which is binding in the presence of a surplus of unlabelled diazepam  $(3\times10^{-6}\,\mathrm{M})$ . By using a benzodiazepine instead of a  $\beta$ -carboline to define nonspecific binding, we excluded interference from presumed ' $\beta$ -carboline sites', unrelated to benzodiazepine receptors. Scatchard analyses of saturation data yielded apparently straight lines (Fig. 1), indicating binding to single classes of sites having affinity constants of  $0.4-0.8\,\mathrm{nM}$ . The values obtained for  $K_D$  are in reasonable agreement with the  $K_I$  value = 1.4 nM for inhibition by  $\beta$ -CCM of <sup>3</sup>H-flunitrazepam (<sup>3</sup>H-FNM) binding in rat cerebellum<sup>7</sup>.

The number of binding sites for <sup>3</sup>H-β-CCM in crude rat cerebellar membranes averaged 56±5 pmol per g tissue  $(\text{mean} \pm \text{s.e.m.}, n = 4)$  which is only 20% less than the number of  $^{3}$ H-FNM binding sites (71 ± 6 pmol per g tissue; mean ± s.e.m., n=3). In the rat hippocampus, however, the number of binding sites for  ${}^{3}\text{H-}\beta\text{-CCM}$  (63 ± 2 pmol per g tissue) was 42% less than that of  ${}^{3}H$ -FNM binding sites (108 ± 4 pmol per g tissue; mean ± s.e.m., n = 3). This is similar to the situation for the radioligand <sup>3</sup>H-PrCC, which was interpreted as <sup>3</sup>H-PrCC binding preferentially to a  $BZ_1$  benzodiazepine receptor subclass<sup>7</sup>. The pharmacological selectivity of <sup>3</sup>H-β-CCM binding sites was very similar to that of <sup>3</sup>H-diazepam and <sup>3</sup>H-PrCC binding sites<sup>1,6</sup> only those agents that act on benzodiazepine receptors inhibit specific <sup>3</sup>H-β-CCM binding and do so at appropriate concentrations (Table 1). These results, together with the chemical similarity of <sup>3</sup>H-β-CCM to the more closely examined <sup>3</sup>H-PrCC<sup>6,7</sup>, suggests that <sup>3</sup>H-β-CCM labels brain BZ receptors, probably with preference for the BZ<sub>1</sub> receptor subclass.

Our main purpose here was to investigate whether  ${}^{3}$ H- $\beta$ -CCM binding was sensitive to GABA receptor stimulation.  $\beta$ -CCM was selected for investigation because it produces pentylenetetrazole-like cloniotonic convulsions in 40–50% of male mice when administered alone in doses which occupy CNS benzodiazepine receptors (10–30 mg per kg intraperitoneally (i.p.), unpublished data). The homologue ester,  $\beta$ -CCE, hardly ever induces convulsions in rats or mice. However,  $\beta$ -CCE enhances the convulsant actions of pentylenetetrazol and bicuculline (a proconvulsant action; refs 12–14 and our unpublished observations). Pharmacological effects of PrCC are not easily determined because very little of this agent reaches the brain after i.p. administration; intravenous administration of 50–200 mg per kg does not produce convulsions in male mice (unpublished results).

Fig. 1 Saturation of specific binding of <sup>3</sup>H-β-CCM and <sup>3</sup>H-flunitrazepam (0.06-8 nM) in rat brain membranes. O, Specific <sup>3</sup>H-β-CCM binding; , specific binding of <sup>3</sup>H-β-CCM in the presence of muscimol (10<sup>-5</sup> M); • nonspecific <sup>3</sup>H-β-CCM binding; • specific <sup>3</sup>H-flunitrazepam binding. Binding assays were done essentially as described for <sup>3</sup>H-PrCC<sup>6</sup>. Crude membranes: hippocampus (a) or cerebellum (b) were rapidly removed and homogenized in  $2 \times 10 \text{ ml}$ ice-cold KH<sub>2</sub>PO<sub>4</sub> (25 mM), pH 7.1, for a few seconds in an Ultra-Turrax homogenizer. The homogenate was centrifuged for 15 min at 48,000g and the pellet resuspended (500 ml per g of original tissue) in another portion of ice-cold buffer. Duplicate aliquots (2.5 ml) of this crude membrane suspension (~90 mg protein per g original tissue) were incubated



subtracting nonspecific binding, which is binding in the presence of diazepam (final concentration  $3 \times 10^{-6}$  M) from total binding. After incubation the samples were filtered through Whatman GF/C glass fibre filters and washed immediately with  $3 \times 5$  ml of ice-cold assay buffer. Radioactivity on the filters was determined by conventional liquid scintillation counting in mini-vials.  ${}^{3}$ H-flunitrazepam (85 Ci mmol<sup>-1</sup>; NEN) binding assays were conducted in a volume of 1.1 ml using 2 mg of original tissue. c, Washed membranes. In experiments designed to investigate the effect of GABA on benzodiazepine receptor binding, an extensively washed, unfrozen tissue preparation was used. Brain tissue (100-1,000 mg) was homogenized using an Ultra-Turrax homogenizer in  $2 \times 10$  ml ice-cold 100 mM Tris-citrate, pH 7.1, and centrifuged for 10 min at 48,000g. The pellet was washed five times in 20 ml Tris-citrate buffer, and each time homogenized and centrifuged as sefore. The final pellet was resuspended (500 ml per g of original tissue) in Krebs-phosphate buffer as indicated ( $\sim 50$  mg of protein per g original tissue). Binding assays were done as described for crude membranes, except that the incubation time was restricted to 20 min at  $0^{\circ}$ C. The Krebs-phosphate buffer contained (final concentration, mM) NaCl (122), KCl (4.8), CaCl<sub>2</sub> (0.97), MgSO<sub>4</sub>,  $7H_2$ O (1.2) and Na<sub>2</sub>HPO<sub>4</sub> (16). Values in a, b and c are from three single experiments, which were all repeated with similar results. Respective  $K_D$  values are shown. B/F, the proportion bound to free radioligand, is expressed as c.p.m. per assay/c.p.m. per assay.

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We found that specific binding of <sup>3</sup>H-β-CCM to rat cerebral cortex membranes was reduced in the presence of the GABA agonist muscimol, a finding in clear contrast to the enhancement of <sup>3</sup>H-diazepam binding (Table 2). Muscimol, GABA and the anomalous GABA agonist piperidine-4-sulphonic acid11, similarly reduced specific binding of <sup>3</sup>H-β-CCM in rat cerebellar membranes. The effects of muscimol and GABA, and to a lesser extent of piperidine-4-sulphonic acid (data not shown), were reduced by bicuculline (Table 3). Bicuculline is believed to be a specific GABA antagonist and the results thus indicate that GABA and muscimol reduce <sup>3</sup>H-\beta-CCM binding at least partially by an effect on the GABA receptors. The affinity rather than the number of <sup>3</sup>H-\beta-CCM binding sites was reduced by muscimol (Fig. 1c).  $\beta$ -CCM did not inhibit high-affinity <sup>3</sup>Hmuscimol binding to presumed GABA receptors (unpublished results).

Table 1 Inhibition of specific <sup>3</sup>H-β-CCM binding in rat cerebellum

Inhibitor	IC <sub>50</sub> (nM)
Lorazepam	1
β-CCE	1.5
PrCC	1.5
β-CCM	3
Diazepam	20
CL 218872	50
Norharman	2,000
Ro 5-4864	13,000
Ro 5-3663	200,000
Ethanol	$\sim 2 \times 10^6$
Meprobamate	>30,000
Pentobarbital	>30,000
Diphenylhydantoin	>30,000
Theophylline	>30,000
Strychnine	>30,000
Picrotoxin	>30,000
Pentylenetetrazole	>30,000
Methysergide	>30,000

Crude membranes were used (see Fig. 1 legend) at a concentration of 5 mg original tissue per 2.675 ml of assay; control specific binding was  $6,100\pm250$  c.p.m. per assay (mean  $\pm$  s.e.m., n=5), nonspecific binding was  $600\pm40$  c.p.m. per assay (mean  $\pm$  s.e.m., n=5).  $^3$ H- $\beta$ -CCM was used at a concentration of 0.35 nM, specific activity 76 Ci mmol (NEN). Each IC<sub>50</sub> value shown is the mean of two independent determinations, each obtained by adding five to six concentrations of the agent to duplicate assays. The concentration causing 50% inhibition of specific binding (IC<sub>50</sub> value) was determined graphically. By pairing the two determinations obtained for each of the nine inhibitory agents, the standard deviation was estimated as 25% of the mean value.

Table 2 Effects of muscimol on specific radioligand binding to rat cerebral cortex

		Non-	Specific bindi	ng (c.p.m. per	assay)
Radioligand	Concentration (nM)	specific binding (%)	Without muscimol	With muscimol (10 <sup>-5</sup> M)	%
<sup>3</sup> H-diazepam <sup>3</sup> H-PrCC <sup>3</sup> H-β-CCE <sup>3</sup> H-β-CCM	0.34 0.35 0.29 0.32	21 29 12 17	$517 \pm 3$ $2,712 \pm 210$ $1,686 \pm 130$ $902 \pm 6$	$1,087 \pm 7$ $3,430 \pm 62$ $1,534 \pm 19$ $746 \pm 2$	210* 126† 91 83*

Tris-citrate buffer was used both for washings and assays; for further details see Fig. 1 legend. Nonspecific binding is shown as a % of total binding in the group 'without muscimol'. Absolute values for nonspecific binding in the group 'with muscimol' were indistinguishable from those in the group 'without muscimol'.  $^3$ H-PrCC is  $^3$ H-propyl  $\beta$ -carboline-3-carboxylate (40 Ci mmol $^{-1}$ ; A/S Ferrosan). Lower values from nonspecific binding were obtained using fresh batches of  $^3$ H-PrCC, for example, NET 703 (NEN).  $^3$ H- $\beta$ -CCE is ethyl  $\beta$ -[6(n)- $^3$ H]carboline-3-carboxylate (24.6 Ci mmol $^{-1}$ ). Values shown are for a single experiment, which was repeated with similar results. Specific binding is given as mean  $\pm$ s.e.m. of three values.

\* P < 0.001; † P < 0.05.

Table 3 Muscimol and GABA reduce specific cerebellar binding of  ${}^{3}$ H-β-CCM; antagonism by bicuculline

	,	-	
Expt	Addition	Specific binding of <sup>3</sup> H-β-CCM (c.p.m. per assay; mean±s.e.m. (n))	% Of control
	Vehicle (control)	$3,225 \pm 57$ (8)	100
	Muscimol (10 <sup>-6</sup> M)	$2,305 \pm 124 (4)$	71*
	Muscimol (10 M)	2,500	
	+ bicuculline $10^{-4}$ M	$2.673 \pm 110(4)$	83+
(1)	Muscimol $(3 \times 10^{-6} \text{ M})$	$2.501 \pm 61$ (4)	78*
(1)	Muscimol $(3 \times 10^{-6} \text{ M})$	2,501 201 (1)	
	+ bicuculline (10 <sup>-4</sup> M)	$2.979 \pm 59$ (4)	92†
	Bicuculline (10 <sup>-4</sup> M)	$3.063 \pm 174 (4)$	95
	Piperidine-4-sulphonic acid	5,005 = x / 1 (1)	, ,
	$(10^{-4} \mathrm{M})$	$1.773 \pm 40  (4)$	55*
	(10 M)		
	Vehicle (control)	$5,656 \pm 89  (6)$	100
	GABA $(10^{-6}  \text{M})$	$4,770 \pm 50  (3)$	84‡
	GABA $(10^{-6} \mathrm{M})$		
	+ bicuculline (10 <sup>-4</sup> M)	$5,520 \pm 110(3)$	98§
	<b>GABA</b> $(3 \times 10^{-6} \text{ M})$	$4,700 \pm 20$ (3)	83*
(2)	GABA $(3 \times 10^{-6} \text{ M})$		
	+ bicuculline (10 <sup>-4</sup> M)	$5,210 \pm 40$ (3)	93
	GABA $(10^{-5} \text{ M})$	$4,350 \pm 50$ (3)	77*
	GABA $(10^{-5} \text{ M})$		
	+ bicuculline (10 <sup>-4</sup> M)	$4,880 \pm 30$ (3)	87
	GABA $(10^{-4} \text{ M})$	$4,510 \pm 30$ (3)	80*
	GABA (10 <sup>-4</sup> M)		
	+ bicuculline (10 <sup>-4</sup> M)	$4,060 \pm 90$ (3)	73§
	Bicuculline (10 <sup>-4</sup> M)	$5,606 \pm 113$ (6)	99
	· · · · · ·		

Washed rat cerebellar membranes were resuspended in Krebsphosphate buffer for assays using 0.37 nM  $^3$ H- $\beta$ -CCM (76 Ci mmol $^{-1}$ ) as described in Fig. 1 legend. The amounts of original tissue used in experiments 1 and 2 were 5 and 8 mg, respectively, per 2.675 ml of assay. Nonspecific binding in the presence of diazepam ( $3 \times 10^{-6}$  M) was 14% of total binding.

\* P < 0.001; ‡ P < 0.01 compared with control (Student's *t*-test). † P < 0.01; § P < 0.04; || P < 0.001 compared with GABA or muscimol alone (Student's *t*-test).

In another series of experiments we investigated the effect of muscimol on inhibition of specific  ${}^3\text{H-}\beta\text{-CCM}$  binding by flunitrazepam. The 50% inhibitory concentration (IC<sub>50</sub>) for flunitrazepam was 5.9 nM in the absence of muscimol, compared with 2.7 nM in the presence of muscimol ( $10^{-5}$  M) (washed rat cerebellar membranes were used, as described in Fig. 1 legend). On the other hand, inhibition of  ${}^3\text{H-}\beta\text{-CCM}$  binding by unlabelled  $\beta\text{-CCM}$  was slightly reduced by muscimol (IC<sub>50</sub> of 1.3 and 1.7 nM, respectively, in the absence and presence of muscimol ( $10^{-5}$  M)). This experiment shows that those benzodiazepine receptors to which  ${}^3\text{H-}\beta\text{-CCM}$  bind increase their affinity for a benzodiazepine by GABA receptor stimulation and that  ${}^3\text{H-}\beta\text{-CCM}$  binding sites are indeed coupled to GABA receptors.

Our results are in agreement with the notion that  $\beta$ -carboline-3-carboxylic acid esters bind to benzodiazepine receptors (presumably the BZ1 receptor subclass) and that these benzodiazepine receptors are coupled to GABA receptors. The conformational change in benzodiazepine receptors induced by GABA, however, affects the binding affinity of a convulsive  $\beta$ -carboline in a manner exactly opposite to the affinity of benzodiazepines. Thus the convulsive  $\beta$ -carboline and anticonvulsant benzodiazepines recognize benzodiazepine receptors in different ways. It is tempting to speculate that the sensitivity to GABA reflects 'agonist/antagonist' potentials of benzodiazepine receptor ligands (see also ref. 8). Furthermore, an intermediate class of ligands may exist, the binding of which is mainly unaffected by GABA and which lack obvious pharmacological effects when given alone. The arguments presented here rely mainly on observations made with  $\beta$ -CCM; further studies are needed, ideally including more efficient and biologically stable convulsive ligands.

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#### ACh release from osmotically shocked synaptosomes refilled with transmitter

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It is still uncertain whether or not acetylcholine (ACh) released at the synapse is derived from vesicles. We have recently described a sensitive chemiluminescent technique which allows continuous measurement of ACh release from tissue slices or synaptosomes incubated in the reaction mixture 1,2. From the luminescence obtained when synaptosomes are opened in the reaction mixture by a single freezing and thawing we estimated that 30% of the total ACh of Torpedo electric organ synaptosomes is cytoplasmic. The vesicular ACh compartment which resists freezing and thawing requires a detergent to be liberated. These estimations showed that the ACh compartment considered as cytoplasmic was readily released from stimulated synaptosomes3. If we are dealing with a real, soluble cytoplasmic ACh compartment, it should in principle be possible to recover, in controlled ionic conditions, a calcium-dependent ACh release from synaptosomes emptied by an osmotic shock, and refilled at the desired ACh concentration. We show here that this is the case. These results suggest the possibility of a non-vesicular release of ACh from the nerve terminal.

Torpedo electric organ synaptosomes were prepared4.5 and concentrated in a physiological solution of 1.1 osmol. After inhibiting acetylcholinesterase using phospholine  $(0.8 \times 10^{-3})$  for 30 min), the synaptosomes were given an osmotic shock in a large volume of hypotonic solution containing 25 mM K<sub>2</sub>SO<sub>4</sub>, 10 mM Tris-HCl pH 6.9, 0.3 mm ATP, 0.3 mM MgCl<sub>2</sub> and a range of ACh concentrations (0-40 mM). Glycine was substituted for ACh at the lower ACh concentrations. After 30 min, the shock period was ended by adding a KCl solution (300 mM

Table 1 Ionophore plus Ca2+ required for ACh release

Ionophore A23187 (without Ca <sup>2+</sup> )	ACh release (amplitude of trace in pmol) 1.25±0.42
Ca <sup>2+</sup> (without ionophore A23187)	$3.00 \pm 0.5$
Ionophore A23187 + Ca <sup>2+</sup>	(3) 11.2±2.7 (12)

The ACh release induced by ionophore + Ca2+ is significantly different from that in non-release conditions, in which either calcium or ionophore are omitted: 0.01 < P < 0.001. No. of determinations is shown in parentheses.

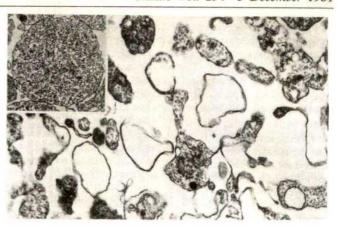


Fig. 1 Synaptosomal sacs. The sacs are either empty or contain glycogen-like granules. Synaptic vesicles are lost during preparation. ×30,800. The insert shows for comparison a synaptosome before shock. Note the numerous synaptic vesicles and the difference in size. ×10,450.

final concentration) which also contained ACh and glycine to maintain the concentrations of these substances. After 30 min, the refilled synaptosomal sacs, equilibrated with ACh and KCl, were centrifuged (27,000g, 30 min), resuspended and sedimented again, before placing them in an 'external medium' consisting of 300 mM NaCl, 25 mM Na2SO4 and 10 mM Tris-HCl pH 8.6. (The alkaline pH necessary for the luminescent reaction did not affect ACh release, as we had previously ensured that the electrical discharge of the tissue remained constant for 3 h at that pH2.) The refilled synaptosomal sacs were resuspended in 100 µl of external medium; 10 or 20 µl were used per assay (this gives ~1 nmol entrapped ACh when the sacs are filled with 40 mM ACh). Considering that these sacs derive from 400 mg of tissue, and that the initial synaptosomal ACh content was  $\sim$ 15 nmol, the yield of this procedure is  $\sim$ 6%.

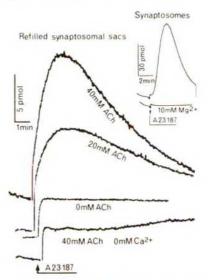


Fig. 2 ACh release from refilled synaptosomal sacs. The synaptosomes were osmotically shocked at 20 °C and refilled with a range of ACh concentrations. The release of ACh triggered by the calcium ionophore A23187 (3.8 µM) increased in proportion to the indicated ACh concentration used to refill the sacs. The omission of calcium greatly reduced ACh release. The insert shows the release of ACh from intact synaptosomes containing ~1 nmol ACh (similar to that from synaptosomal sacs filled with 40 mM ACh). The release from synaptosomes was much reduced (lower trace) when magnesium replaced calcium. The release curve for synaptosomes is comparable to that for refilled synaptosomal sacs. The reaction mixture consisted of 470 µl external medium, with or without 10 mM CaCl2, 2.6 units choline oxidase (Wako), 10 µg horseradish peroxidase type II (Sigma), 10 µl luminol (1 mM; Merck) and 1.7 units acetylcholinesterase from Electrophorus (Boehringer) passed through Sephadex G-50.

The amount of entrapped ACh was, as expected, proportional to the ACh concentration used to refill the shocked synaptosomes. This was verified by disrupting the sacs in the reaction mixture with Triton X-100. Most (85%) of the entrapped ACh was liberated by freezing and thawing the refilled synaptosomal sacs, in contrast to intact synaptosomes where most of the ACh (70%) is bound to synaptic vesicles, resists freezing and thawing and requires a detergent or an osmotic shock to be liberated. A morphological analysis of the refilled synaptosomal sacs showed that the synaptosomes had indeed lost their structure during the procedure (see Fig. 1). The sacs lost their synaptic veiscles but not all were empty as some retained a reticulum-like network and glycogen-like granular material.

Substantial ACh release from the refilled synaptosomal sacs was observed after calcium influx had been triggered by the calcium ionophore A 23187. This release of ACh increased in proportion to the internal ACh concentration (Figs 2, 3a). For comparison, the release of ACh from intact synaptosomes containing ~1 nmol ACh (similar to the sample of sacs filled with 40 mM ACh) is shown in Fig. 2 insert. For both cases the release curves were very similar. To measure the proportion of ACh content of the sac that was released as a result of the calcium influx, we estimated ACh content before and after triggering the release by disrupting the sacs with Triton X-100. Their ACh content declined by as much as 50% in 10 min, at which time the release curve still represents 25% of its maximum amplitude. The specificity for ACh of the release process was tested by filling the sacs with choline. In this case there was a 70% reduction in release, indicating a clear preference for ACh (see Fig. 3a). The calcium dependency of transmitter release from synaptosomal sacs refilled with 40 mM ACh is shown in Fig. 2 (lower trace). There was no release when ionophore was added in the absence of calcium. When extracellular calcium was increased, the ionophore became efficient (Fig. 2, upper trace). The amplitude of ACh release triggered by the ionophore clearly increased when the extracellular calcium concentration was increased (Fig. 3b).

Gramicidin D, which triggers ACh release from intact synaptosomes<sup>3</sup>, also acted on the synaptosomal sacs, which released ACh in proportion to their content. In both cases the release of

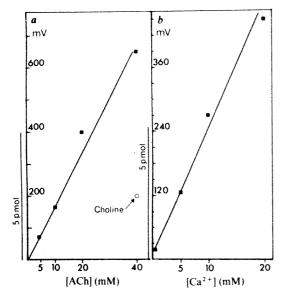


Fig. 3. a, Effect of internal ACh concentration on its release from synaptosomal sacs. The amplitude of ACh release curves is plotted for the various ACh concentrations used to refill the shocked synaptosomes. ACh release was triggered by the calcium ionophore as in Fig. 2. Note that when choline was substituted for ACh (o), release was much reduced. b, Effect of external calcium on amplitude of ACh release. Synaptosomal sacs filled with 40 mM ACh were treated with ionophore A23187 at various extracellular calcium concentrations. The amplitude of release curves increased in proportion to extracellular calcium concentration

ACh was much depressed when magnesium was substituted for calcium.

To determine whether the pH gradient (acid in the sac) was involved in the observed ACh release, we repeated the experiment shown in Fig. 2 at a constant pH(7.9) inside and outside the sac. When ionophore A23187 was added in the absence of calcium, there was no significant ACh release but this was triggered by the subsequent addition of calcium. If calcium was added in the absence of ionophore, only a small amount of ACh was released and the subsequent addition of ionophore A23187 triggered the usual ACh release (Fig. 2). Table 1 gives the mean amplitude of the ACh release curve compared with that for non-release conditions. It is clear that ACh release from the sacs is calcium dependent, even at constant pH.

In conclusion, we were able to obtain a calcium-dependent ACh release from synaptosomal membrane sacs filled with ACh in controlled ionic conditions. The release of ACh increased in proportion to the internal ACh content and to the external calcium concentrations. This model might serve as a means of studying release of cytoplasmic ACh recently measured for intact synaptosomes3. We do not exclude the possibility that a vesicular mechanism coexists with the one described here, which cannot be attributed to synaptic vesicles. The intramembrane particles of the presynaptic membrane, which undergo important changes during synaptic activity<sup>6,7</sup>, may play a prominent part in release of cytoplasmic ACh.

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#### Persistent behavioural effect of apomorphine in 6-hydroxydopamine-lesioned rats

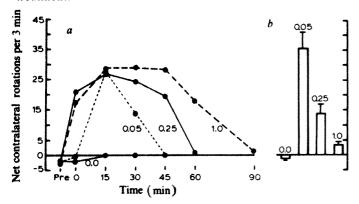
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The rotational (circling) behaviour of rodents with unilateral nigrostriatal damage has been used extensively to investigate nigrostriatal function and the action of dopaminergic compounds<sup>1</sup>. Rats lesioned by unilateral microinjection of the catecholaminergic neurotoxin, 6-hydroxydopamine OHDA), spontaneously exhibit slight ipsilateral (towards the lesioned side) rotation. Systemic challenge with the dopamine receptor agonist apomorphine induces active contralateral rotation, apparently as a result of supersensitivity in the lesioned hemisphere2,3. We report here that apomorphine treatment also results in an extraordinarily persistent change in spontaneous (undrugged) rotation. After one treatment with 50 µg per kg apomorphine, exposure to the rotation environment resulted in a brief, intense burst of contralateral rotation. This behaviour, apparent for months after drug treatment, did not fully develop until 2 weeks after treatment and was never observed in lesioned, but otherwise drug-naive rats. The latent apomorphine effect, unlike the acute effect, was inversely related to dose over the range tested. The behaviour was relatively specific for the environment in which drug treatment had occurred, suggesting a role for learning, but it is difficult to reconcile the inverse dose-response function, lack of recency effect and inability to induce an analogous phenomenon using (+)amphetamine with this explanation.

Male Sprague-Dawley rats (180-240 g) were lesioned unilaterally by injection of 8 µg (base) 6-OHDA hydrobromide (Aldrich). Two to four weeks later rats were injected subcutaneously (s.c.) with saline or apomorphine HCl (0.05, 0.25 or 1.0 mg per kg; Merck). Each rat was placed in a clear plastic hemispherical bowl (26 cm diameter) and observed for 3-min periods before injection, immediately after injection, and periodically thereafter until rotation had slowed to near zero. Total 360° turns in each direction were counted during each observation period. Net contralateral turns were calculated by subtracting the number of ipsilateral turns from the number of contralateral turns. Rats which made fewer than 10 net contralateral turns in one of the observation periods after apomorphine treatment were considered to be only poorly lesioned (<15% of subjects) and their data were disregarded.

Before injection, all groups exhibited slight ipsilateral bias (Fig. 1a). Shortly after apomorphine injection, rapid contralateral rotation began, in the virtual absence of ipsilateral turning. Consistent with previous reports<sup>2,4</sup>, this acute effect of apomorphine was dose-related with respect to duration but dose-independent with respect to peak rate. When placed in the bowl 2 weeks after treatment, in a 3-min observation period rats that had received saline continued to show slight ipsilateral bias whereas apomorphine-treated rats showed almost exclusive contralateral rotation, which was inversely related to the previously administered dose (Fig. 1b). In the case of the group receiving the lowest dose, the rate of rotation in the 3-min observation period 2 weeks after drug treatment was, remarkably, higher than the peak rate observed immediately after drug administration. The animals rotated in tight circles, most rapidly in the first minute, and soon slowed, usually stopping by the end of the 3-min test; no ipsilateral turns were made. Some of the rats treated with saline, 0.05 or 1.0 mg per kg apomorphine were repeatedly tested for rotation at 2-8-week intervals with no further drug treatment. Behaviour of these subgroups was consistent with that shown in Fig. 1b for as long as it was tested: saline-treated rats continued to exhibit slight ipsilateral bias. and rats treated with 0.05 mg per kg apomorphine continued to exhibit rapid contralateral rotation, up to 9 months after treatment. Rats treated with 1.0 mg per kg apomorphine continued to exhibit only slight contralateral rotation up to 14 weeks after treatment.



Acute and long-term rotational response to saline or apomorphine (0.05, 0.25 or 1.0 mg per kg) in 6-OHDA-lesioned rats. 6-OHDA (8 µg base in 4 µl saline and 0.1% ascorbic acid) was injected at a rate of 0.33 µl min<sup>-1</sup> into 180-240-g rats anaesthetized with pentobarbital. Coordinates (with incisor and ear bars levelled) were 4.2 mm posterior and 1.4 mm lateral from bregma, 8.0 mm deep from skull surface. A single s.c. injection of apomorphine HCl or saline was administered 2-4 weeks post-lesion (n = 10-14 per group). a, Mean net contralateral rotations made in 3-min observation periods before injection (Pre), and at the indicated times after injection of saline or apomorphine. b, Mean +s.e.m. net contralateral rotations made 2 weeks after saline or apomorphine treatment when rats were placed in the rotation bowl for a 3-min observational period. All groups except the salinetreated showed a significant difference in rotation 2 weeks after treatment compared with spontaneous rotation before treatment (paired Student's t-tests, P < 0.001 in each case). The effect of each dose of apomorphine measured 2 weeks after treatment was significantly different from the effect of each of the other doses (one-way analysis of variance followed by Student-Newman-Keuls test, P < 0.05 in each case).

Table 1 Persistent rotational response to apomorphine HCl (0.05 mg per kg) in 6-OHDA-lesioned rats as a function of time interval between drug administration and re-exposure to rotation environment

No. of animals	Acute rotational response*	Interval between injection and test (days)	Latent rotational response†
11	$28.3 \pm 4.0$	1	$9.5 \pm 3.0$
8	$27.2 \pm 3.9$	4	$11.4 \pm 4.1$
7	$27.8 \pm 1.9$	7	$12.0 \pm 2.1$
7	$27.1 \pm 2.5$	14	$37.8 \pm 7.1 \ddagger$
9	$26.9 \pm 3.0$	28	$33.7 \pm 7.5 \ddagger$

- \* Contralateral rotations per 3 min beginning 15 min post-injection (no insilateral turns were made).
- † Net contralateral rotations per 3 min on the days indicated (all groups averaged <1 ipsilateral turn).
- $\ddagger$  Significantly different from 1, 4 and 7 day groups (P < 0.025; one-way analysis of variance and Student-Newman-Keuls test).

An additional group of lesioned rats (n = 5) was injected with saline before being placed in the bowl for 48 min; after 2 h they were injected with 0.05 mg per kg apomorphine and observed to rotate in their home cages. This group failed to exhibit contralateral rotation when tested in the bowl from 2 to 24 weeks after treatment. Some of these subjects occasionally showed sudden contralateral rotation when returned from the bowl to their home cages. This stimulus (environmental) specificity was tested further in two groups of lesioned rats. Two weeks post-lesion, rats were treated with 0.05 mg per kg apomorphine on three consecutive days. After each injection, subjects were placed in the bowl (n = 5) or in a rectangular  $(20 \times 21 \times 24 \text{ cm})$  opaque plastic box (n = 5) for 48 min. Two weeks after the last drug treatment, rats that had rotated in the bowl were tested for 3 min in the box and, 30 min later, for 3 min in the bowl. Conversely, rats that had rotated in the box were tested first in the bowl, then in the box. Rats showed significantly greater rotation in the environment in which they had previously received drug treatment than in the novel environment (mean =  $36.6 \pm 9.6$  to  $3.3 \pm$ 2.3 net contralateral rotations per 3 min; P < 0.02; paired Student's t-test).

As the stimulus specificity of the behaviour suggested a role for learning, five groups of lesioned rats were tested for recency effect. Two weeks post-lesion, rats were treated once with 0.05 mg per kg apomorphine and were observed to rotate in the bowl as described for the dose-response experiment. Rats were then placed in the bowls for a 3-min observation period 1, 4, 7, 14 or 28 days after drug treatment. Each rat was thus re-exposed to the rotation bowl only once after drug treatment. Groups tested 14 or 28 days after drug treatment showed significantly greater contralateral rotation than those tested at *shorter* intervals after treatment (Table 1).

Ungerstedt<sup>5,6</sup> reported that some 6-OHDA-lesioned rats developed 'explosive' contralateral rotation at ~2 weeks post-lesion. As the aetiology was unknown, this was termed 'paradoxical' rotation. We consider that paradoxical rotation and the latent, persistent effect of apomorphine shown here are, in fact, the same phenomenon. We have never observed paradoxical rotation in over 200 lesioned, but otherwise drugnaive, rats at 2 to 30 weeks post-lesion. In rats that had been lesioned 30 weeks previously, it was still possible to induce paradoxical rotation by apomorphine treatment.

The stimulus specificity of paradoxical rotation suggests that it may be an extraordinary example of associative learning. If so, it is difficult to explain the inverse dose relationship and lack of recency effect. Furthermore, associative learning alone would not predict any pharmacological specificity, that is, any compound inducing acute rotation, regardless of direction, might be expected to induce a similar latent, persistent rotation. LSD, which, like apomorphine, induces acute contralateral rotation<sup>7</sup>, also resulted in paradoxical rotation after a single treatment (unpublished data). However, we have been unable to induce an analogous phenomenon with either single or repeated

(+)amphetamine treatments, which induced acute ipsilateral rotation.

Just as associative learning does not seem to fully explain our data, we know no previously described effect of apomorphine that would. The effects of low doses of apomorphine have been associated with presynaptic activity8-10. While apomorphineinduced rotation in 6-OHDA-lesioned rats has usually been attributed to direct action on supersensitive striatal neurones, a role in this behaviour has recently been ascribed to drug action in the substantia nigra itself11. It may be that in rats having nigrostriatal 6-OHDA lesions, the presynaptic effect of a small dose of apomorphine is greatly exaggerated over that in intact rats. Paradoxical rotation may then be the result of both learning and a persistent biochemical consequence of drug adminis-

Our finding that paradoxical rotation results from a single treatment with apomorphine should serve as a caveat to those who routinely verify lesions behaviourally by administering a 'screening' dose of apomorphine. Paradoxical rotation may, however, prove useful for studying chronic effects of acute phychoactive drug administration, for example, phenomena such as LSD flashbacks12

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#### Multiple tubulin forms are expressed by a single neurone

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Microtubules are a key cytoskeletal constituent of the eukaryotic cell, being involved in mitosis, cell division, cell shape, intracellular transport and motility<sup>1</sup>. They are composed of tubulin, a 110,000-molecular weight  $(M_r)$  protein which is a dimer of 55,000- $M_r$   $\alpha$  and  $\beta$  submits<sup>2</sup>. Multiple forms of both  $\alpha$ and  $\beta$ -tubulin have been demonstrated biochemically by gel electrophoresis<sup>3,4</sup>, column chromatography<sup>5</sup> and peptide maps<sup>6</sup>; immunologically by monoclonal antibodies<sup>7</sup>; and genetically by mapping multiple tubulin genes in the eukaryotic genome There is evidence for multiple tubulin mRNA species 11,12,13, but some of the microheterogeneity may also arise from posttranslational modifications such as phosphorylation14 and glycosylation15. Tubulin microheterogeneity is most dramatic in the brain where it could be the result of cellular heterogeneity or a multiplicity of specific functions within each cell, such as neurite extension and axoplasmic transport<sup>1,16</sup>. Functional specificity has been demonstrated in particular tubulin subunits in other tissues<sup>9,16,17</sup>. To determine whether multiple forms of tubulin coexist in single cells, we have purified and characterized the tubulin of single, isolated sympathetic neurones grown in

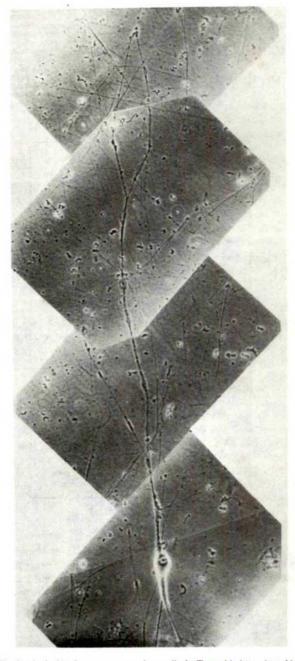
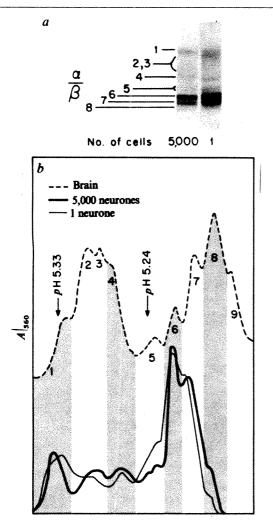


Fig. 1 An isolated neurone grown in a well of a Terasaki plate, viewed by phase-contrast microscopy. Scale bar, 40 µm. Neurones from the superior cervical ganglia of newborn rats were grown as described previously Briefly, the ganglia were mechanically dissociated and the cells plated on to collagen in a medium containing nerve growth factor (NGF). Cultures were kept in a 5% CO2 atmosphere in medium containing 20mM K+. For studies of solitary neurones, Falcon Terasaki tissue culture plates were prepared as follows. The bottom of each well was coated with  $1~\mu l$  of solubilized collagen and the dish exposed to ammonia vapours. The resulting collagen gels provide a more adhesive surface than dried films of collagen<sup>27</sup>. Medium (10 μl) was added to each well, and the dish was sterilized by exposure to UV light. Ganglia were dissociated first with forceps, passed 10 times through a 23G hypodermic needle, then seeded into the dish (<100 cells) in 6 ml of culture medium. In successful platings, each plate had three or four wells with isolated neurones (out of a total of 60 wells), the rest of the wells containing none or only small clumps of neurones. Medium was changed every 3 or 4 days.

primary cell culture18. We show here for the first time that tubulin can be isolated and characterized from a single mammalian neurone. Single neurones exhibit multiple tubulin isoelectric forms, suggesting that structurally different forms of microtubules have functionally different roles in a single cell.

Single neurones grown in microwells of Terasaki plates showed extensive morphological differentiation after 3 weeks in culture. Figure 1 shows a phase-contrast micrograph of a single cell in an isolated well. The cell has well-defined dendritic and

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axonal processes. Each cell was labelled with <sup>35</sup>S-methionine for 18 h and radioactive tubulin isolated by vinblastine precipitation in the presence of unlabelled brain carrier, then subjected to isoelectric focusing and autoradiography. Figure 2 shows that a

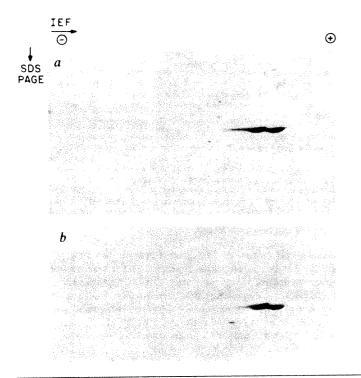
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Fig. 2 Multiple tubulin forms are found in a single neurone. Mature cells (3–4 weeks in culture) were labelled with <sup>35</sup>S-methionine (900–1, 000 Ci mmol<sup>-1</sup>, 6–8 mCi ml<sup>-1</sup>; Amersham) as follows. Mass cultures of 2,000– 5,000 cells were incubated for 18 h in 40 µl of methionine-deficient L-15 culture medium (Gibco) containing 10 µM cold methionine and 1 µM <sup>15</sup>S-methionine. Cultures were collected and sonicated for 3 min in a bath sonicator (Laboratory Supplies, New York) in the presence of 250 µl of 0.24 M sucrose, 10 mM Na-phosphate buffer pH 7.4, 10 mM MgCl<sub>2</sub>, 0.2 mM GTP and 100 µl of brain homogenate prepared from mature rat brain in 2 vol of the same buffer. The resulting homogenates were centrifuged first at 12,000 g for 10 min and then at 100,000 g for 1 h. Vinblastine sulphate (1 mg ml-1) was added to the high-speed supernatants. Samples were incubated at 37 °C for 30 min, then the tubulin aggregates were sedimented at 5,000 g for 5 min. The tubulin-enriched pellets<sup>28</sup> were each suspended in  $50 \,\mu$ l of lysis buffer<sup>29</sup> and  $10 \,\mu$ l aliquots were subjected to isoelectric focusing<sup>29</sup>. Single cells in microwells of Terasaki tissue culture plates were incubated in 10  $\mu$ l of methionine-deficient medium containing 10  $\mu$ M <sup>35</sup>S-methionine for 18 h. They were then collected in 50  $\mu$ l of the buffer used for the mass cultures plus 0.5% Triton X-100 and were sonicated in the presence of 20 µl of brain homogenate. (The addition of Triton X-100 did not interfere with subsequent experimental procedures.) Vinblastine precipitation and isoelectric focusing were performed as above, except that all the material obtained from a single cell was applied to one isoelectric focusing gel. The final pH gradient was determined by eluting 2-mm portions of a blank gel in 10ml H<sub>2</sub>O. Gels were stained, dried and exposed to Kodak X-R5 X-ray film and Dupont Cronex lighting-plus intensifying screens<sup>30</sup> for weeks, depending on the amount of label present. The numbers identifying the various isotubulin bands were assigned by alignment with Coomassie brilliant blue staining of the rat brain tubulin carrier. a Autoradiogram of the isoelectric focusing gels; b, densitometric tracings of the autoradiogram shown in a. Shaded areas were divided according to the peaks of the isotubulin bands of the sympathetic neurones and aligned with the co-migrating brain carrier. Nine isotubulins are observed in the brain tubulin; 1-4 comprise the classically defined  $\alpha$ -tubulin subunit, and 5-9 the β subunit<sup>3</sup>. The co-electrophoresed cold carrier brain tubulin and radiolabelled cultured neuronal tubulin show different proportions of the various isotubulins, which shows that the isoelectric focusing patterns are not artefacts of discontinuities in the ampholine pH gradient. Previous reports have established that brain tubulin microheterogeniety does not arise from proteolysis during purification. As the carrier brain isotubulin pattern is unaltered in the presence or absence of sympathetic neurone extract (data not shown), degradation during the purification procedure by proteases derived from the neurones is unlikely. Densitometric traces analysed by planimetry revealed  $67\pm4\%$   $\beta$ -tubulin and  $33\pm4\%$   $\alpha$ -tubulin in the vinblastine precipitates of high-speed supernatants of cultured neurones. When these values are corrected for methionine content (11 for  $\alpha$ , 19 for  $\beta$ )<sup>31–33</sup> of the tubulin, we obtain a ratio for  $\alpha/\beta$  close to the 1:1 ratio of  $\alpha$ - to  $\beta$ -tubulin

single neurone expresses up to seven isotubulins in a pattern very similar to that expressed by mass cultures of 5,000 cells.

found in nonradioactive precipitants from rat brain.

We performed several experiments which demonstrated that the isotubulin patterns obtained by one-dimensional isoelectric



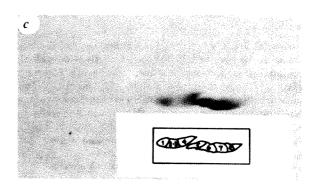
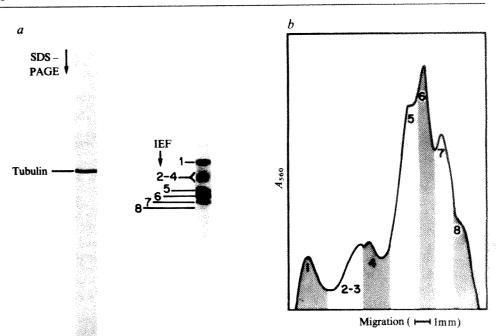


Fig. 3 Two-dimensional polyacrylamide gel electrophoresis (PAGE) of tubulin purified by vinblastine precipitation. The isoelectric focusing gels of mass culture tubulin (Fig. 2) were subjected to electrophoresis in SDS on a gradient of 7-17% polyacrylamide in the second dimension? Gels were stained with Coomassie brilliant blue, dried and autoradiographed as described in Fig. 2 legend. a, Coomassi brilliant blue stain of the cold carrier. The contribution of sympathetic neurone tubulin to the stained cold carrier brain tubulin is at the most 5-10% (0.5  $\mu$ g from the cultures/5-10  $\mu$ g of cold carrier). b, Autoradiogram of the 35S-labelled neuronal tubulin of the same gel shown in a; 24 h exposure. c, Same as b, but a 4-h exposure; enlarged 3 ×. Inset, spots were numbered by visual inspection. The relative intensities of the spots correspond to the intensities of the isoelectric focusing bands numbered in Fig. 2.

Fig. 4 Tubulin microheterogeneity in sympathetic neurones is independent of the method of purification used. 35S-methionine-labelled tubulin was isolated by DEAE-cellulose chromatography. Mature cells (3-4 weeks in culture) were labelled with <sup>35</sup>Sculture) were labelled with methionine (100  $\mu$ Ci per plate of 2,000–5,000 cells). Cells from five culture plates were collected and mixed with 1.5 ml of brain homogenate prepared in 2 vol of 0.24 M sucrose, 2.5 mM MgCl<sub>2</sub> and 50 mM Na-pyro-phosphate pH 7.0. Cells were sonicated as in Fig. 2 and centrifuged at 30,000 g for 30 min. The resulting supernatant was subjected to DEAE-cellulose chromatography for tubulin purification3. a, Purified tubulin was subjected to electrophoresis in SDS on a gradient of 7-17% polyacrylamide (SDS-PAGE) as well as to isoelectric focusing (IEF). b, The isoelectric focusing gels were densitometrically scanned and shaded areas divided as in Fig. 2b.



focusing were not due to contamination by material other than tubulin. Isoelectric focusing and subsequent SDS-polyacrylamide gel electrophoresis on a second dimension (Fig. 3) show that there is considerable tubulin purification by vinblastine precipitation. The gel was scanned in seven consecutive vertical paths within the pH range occupied by the various tubulins, and the optical density quantitated by planimetry. Several faint contaminating proteins together comprised 7.4% of the total optical density, and the remaining 92.6% of the label was judged to be contained in tubulin, by the criteria of molecular weight and isoelectric point. No single contaminating protein comprised >1.3% of the total label, and as no single isotubulin band comprised < 8.1% of the total label, it is clear that none of the isotubulin bands seen after isoelectric focusing can be attributed to contaminants. Moreover, the contaminating spots were detected only after a 24-h exposure, at which time the tubulin bands were over-exposed. A shorter (4 h) exposure (Fig. 3c) reveals multiple tubulin spots corresponding to the isotubulin pattern obtained by isoelectric focusing in one direction (compare with Fig. 2; see also ref. 4); in this exposure most contaminating spots could not be detected (data not shown).

To demonstrate further that tubulin microheterogeneity in sympathetic neurones is independent of the method of purification, tubulin was purified from <sup>35</sup>S-methionine labelled mass cultures by DEAE-cellulose chromatography3. This technique gave >96% pure tubulin as judged by SDS-polyacrylamide gel electrophoresis (Fig. 4a). Isoelectric focusing revealed a similar isotubulin pattern to that found for the vinblastine precipitate (Figs 2 and 4a, b) and showed the presence of at least seven isotubulins.

These data indicate that a single neurone is capable of expressing virtually the whole range of isotubulins expressed by mass cultures. Although the tubulin microheterogeneity found in sympathetic neurones is comparable in extent with that previously found in brain<sup>3,4,7,14-16</sup>, the proportions of the various isotubulins in the cultured neurones are different from those found in the whole brain tubulin carrier (Fig. 2b). For example, isotubulins 2 and 3 are greatly enriched in the tubulin of the whole brain. As sympathetic neurones were grown in the absence of non-neuronal cells, this difference could be due to cell or tissue specificity of tubulin forms. Our data cannot determine whether the different proportions of isotubulins in brain and cultured neurones arise from the absence of glia the elimination of many neurone types, or differences between newly synthesized tubulin in the cultures and the pre-existing tubulin pool in brain.

The existence of multiple tubulin forms in a single neurone

suggests two different hypotheses for specificity in microtubule organization and function: (1) The neurone may have various different forms of microtubules, each containing different isotubulin types. These microtubules may have specialized functions or subcellular distributions. In neurones, the  $\alpha$  subunit class is preferentially associated with synaptic membranes<sup>21</sup> and synaptic vesicles<sup>22</sup>. We have preliminary evidence from neurones grown in a three-chamber culture dish (which separates the cell bodies from axons<sup>23</sup>) that there is an enrichment in the soluble  $\beta$ -tubulin class (isotubulins 5-8) in the axoplasm relative to the cytoplasm as analysed by isoelectric focusing. These results are similar to those of Brady et al.24 who found axonal transport of mainly the classically defined  $\beta$ tubulin by two dimensional gel electrophoresis. In sea urchins, using peptide mapping, Stephens<sup>6</sup> showed that the tubulin subunits of cilia, flagella and cytoplasm have some non-identical peptides, indicating differences in primary structure. (2) Each microtubule in the cell may contain all the isotubulin forms, which may differ by having specific binding sites for regulatory molecules and various subcellular organelles. Microtubule functional specificity may thus result not only from structural differences in the tubulin molecule itself but also from differences in the microtubule associated proteins<sup>25</sup>. Changes in composition and activity of microtubule-associated proteins have been observed during brain development<sup>26</sup> with a time course similar to that of changes in tubulin micro-heterogeneity<sup>3,11</sup>. It will be of interest to see whether the structural differences between the various isotubulins affect the binding of specific associated proteins and correlate with specific microtubule functions in the single cell.

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#### E. coli uvrB protein binds to DNA in the presence of uvrA protein

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In vivo and in vitro, the endonucleolytic incision of damaged DNA containing pyrimidine dimers or other bulky lesions requires the action of the uvrA, uvrB and uvrC gene products of Escherichia coli<sup>1-3</sup>. To understand better how the products of these three genes mediate incision, all three genes have been cloned4-10. Although the gene products of the three genes (designated UVRA, UVRB and UVRC) are all required at the early stages of nucleotide excision repair, it is not known whether they act independently or together as part of a multiprotein complex. As the three proteins are involved in the repair of damaged DNA, it is reasonable to expect that under some circumstances all three proteins should bind to DNA, and, in accord with this expectation, previous experiments have demonstrated that UVRA and UVRC bind tightly to singlestranded DNA<sup>11-13</sup>. We have investigated whether UVRB is also a DNA-binding protein and report here a radiochemical purification of UVRB and experiments using this radiolabelled UVRB to measure its binding to DNA. By itself, UVRB does not bind strongly to DNA, but in the presence of UVRA, tight binding of UVRB to single-stranded DNA is observed, thus demonstrating the occurrence of direct interactions between UVRA and UVRB.

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Fig. 1 SDS gels of the preparations of UVRB and UVRA used in DNA binding experiments. Lane 1, autoradiogram of UVRB. 35S-labelled autoradiogram of UVRD. UVRB was prepared from 21 of 35S-L-·S-(1 μCi methionine) maxicells of strain CSR603 carrying the uvrB plasmid pDR1494. pDR1494 carries the uvrB gene and has been described elsewhere Selective lysis 2.18 was followed by chromatography DEAE Biogel (Biorad) and Biogel HT (Biorad) to yield a preparation containing  $23~\mu g~ml^{-1}$  total protein and  $24,000~c.p.m.~ml^{-1}$  in buffer A (50 mM K-MOPS, 100 mM KCl, 1 mM Na<sub>2</sub>EDTA, 10 mM mercaptoethanol and 25% glycerol). Lane 1 shows the autoradiogram of the SDSgel analysis of 50 µl of this preparation. Lane 2, Coomassie blue-stained gel of the UVRA preparation. UVRA was prepared as described for fraction III in ref. 12 and contained 30 µg protein per ml. Lane 2 shows the Coomassie blue staining pattern of the SDS-gel analysis of 50 µl of this preparation. The subunits of E. coli

β,β' =

RNA polymerase were run as molecular weight markers (165,000, 155,000, 90,000 and 40,000), and their positions in the gel are indicated in the left margin.

We have developed the maxicell procedure for specifically labelling plasmid-encoded proteins, and have used this in combination with transposon inactivation to identify and to purify protein products of cloned genes 7.11,12,14-16. By comparing plasmid-encoded proteins synthesized in maxicells with those synthesized by derivatives in which the uvr genes were inactivated by insertion of a transposon, UVRA, UVRB and UVRC have been identified as polypeptides with molecular weights of 114,000, 84, 000 and 70,000, respectively 11,15

UVRB was labelled in maxicells as described previously15 and was partially purified so that no other labelled proteins are

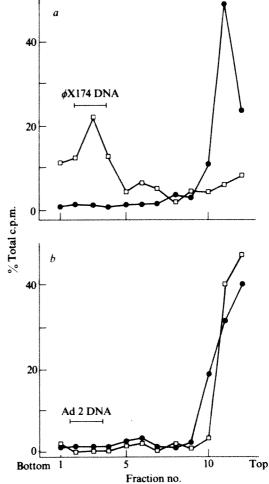


Fig. 2 Binding of radiolabelled UVRB to DNA in the presence of UVRA. 50 µl of the UVRB preparation (Fig. 1, lane 1) is mixed with 0 (●) or 10 µl (□) of the UVRA preparation (Fig. 1, lane 2) with 10 or 0  $\mu$ l buffer A, 7  $\mu$ l 1 M MgSO<sub>4</sub>, 60  $\mu$ l buffer Y (50 mM K-MOPS pH 7.5, 100 mM KCl, 1 mM Na<sub>2</sub>EDTA, 10 mM mercaptoethanol) and 18 µg ФX174 single-stranded circular DNA (a) or adenovirus 2 double-stranded linear DNA (b) in  $60 \mu l$ 10 mM Tris-HCl pH 8.0, 1 mM Na<sub>2</sub>EDTA. Samples were kept at room temperature for 15 min and then applied to 5-20% linear sucrose gradients (5 ml in buffer Y and 20 mM MgSO<sub>4</sub>). The gradients are run for 3h in a Beckman SW50.1 rotor at 45,000 r.p.m. and 25°C after which 0.4 ml fractions are collected and assayed for radioactivity which is plotted above as a function of the gradient fraction. The bar indicates the position of DNA in the gradient.

detected by autoradiography after SDS-gel electrophoresis (Fig. 1). The radiolabelled UVRB was mixed with either singlestranded or double-stranded DNA and sedimented through a sucrose gradient. The results in Fig. 2 show that there is little or no binding to either form of DNA. We then looked for other factors that might cause binding of UVRB to DNA, being particularly interested in learning whether the addition of purified UVRA would have an effect. UVRA was isolated as described elsewhere 12. The Coomassie blue-stained SDS gel (Fig. 1, lane 2) shows that UVRA is the only major stainable protein in this preparation. As seen in Fig. 2, when UVRA is present in the mixture, UVRB co-sediments with the singlestranded DNA. As previous studies showed that UVRA had a high affinity for single-stranded DNA, these results indicate that UVRB probably forms a ternary complex with UVRA and single-stranded DNA, because UVRB co-sediments with ΦX174 single-stranded DNA in the presence, but not in the absence, of added UVRA. This provides a mechanism by which UVRB is in the proximity of damaged parts of a DNA molecule even though by itself the protein does not bind to DNA. These results show that UVRA and UVRB interact with each other and strongly suggest that the two proteins act together rather than independently during nucleotide excision repair. The stoichiometry of this reaction and the role of UVRC and other effectors are under investigation.

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#### **Enrichment of satellite DNA** on the nuclear matrix of bovine cells

#### Lloyd H. Matsumoto

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The skeletal framework of the enkaryotic nucleus is a protein matrix1,2 which maintains the physical shape of the nucleus. Attached to the matrix is a fraction of DNA resistant to deoxyribonuclease I (DNase I) digestion. Ribosomal DNA sequences also are enriched on nuclear matrices from rat liver. The question thus arises whether other repeated DNAs are preferentially attached to the nuclear matrix. This question could be resolved if the repeated DNA was available in sufficient quantity for biochemical studies and differed in its physical or sequence characteristics from the rest of the nuclear DNA. The satellite DNAs of bovine cells meet these criteria. Here we show that nuclear matrices from bovine cells are in fact enriched in satellite DNA.

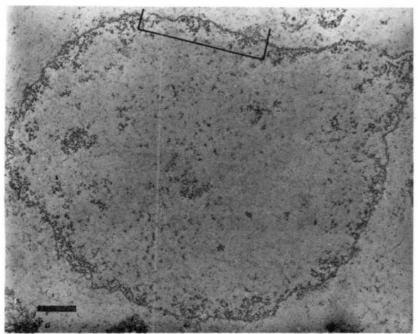
Madin-Darby bovine kidney cells4 were cultured in reinforced Eagle's medium supplemented with 5% fetal calf serum (FCS) and 5% calf serum as previously described<sup>5</sup>. Cells were grown to ~90% confluence on 150-mm Nuncion plates, collected by trypsinization and counted in a haemocytometer. To facilitate nuclear isolation, cells were first incubated for 10 min in a low magnesium buffer: 10 mM Tris-HCl, 0.2 mM MgCl<sub>2</sub> pH 7.4. This and all subsequent nuclear matrix isolation procedures were carried out at 0 °C. Nonidet P-40 (Calbiochem) was added to a final concentration of 1% and the cells were homogenized with 5-10 strokes of a loose-fitting Dounce glass homogenizer. Nuclei were collected by centrifugation at 750g for 8 min and the nuclear pellet resuspended in the low magnesium buffer and again collected by centrifugation. Nuclear matrices were prepared according to the procedure of Pardoll et al.6. Briefly, purified nuclei were resuspended in the low magnesium buffer followed by slow addition of NaCl to a final concentration of 2 M. To this solution was added DNase I (Worthington) to a final concentration of 200 U ml<sup>-1</sup> for 20 min. The matrices were collected from this solution by centrifugation at 2,000g for 15 min. An example of a nuclear matrix isolated in these conditions is shown in Fig. 1.

DNA was extracted from the purified nuclear matrices by resuspending them in 50 mM Tris-HCl, 10 mM NaCl, 10 mM EDTA pH 7.4. T<sub>1</sub> ribonuclease and ribonuclease A (Millipore) were added to final concentrations of 25 U ml-1 and 100 μg ml<sup>-1</sup> , respectively. Matrices were incubated in this solution at 37 °C for 30 min. After RNase treatment, SDS and proteinase K (Boehringer Mannheim) were added to final concentrations of 0.5% and 100  $\mu g$  ml<sup>-1</sup>, respectively; incubation was at 37 °C for an additional 30 min. The residual DNA in this solution was extracted twice with phenol and once with diethyl ether. The solution was adjusted to 0.5 M NaCl and the DNA ethanol-precipitated by the addition of 2 vol 95% ethanol. Typically, the residual nuclear matrices contained ~1% of the total DNA when compared with a theoretical value based on the haploid DNA content of the bovine cell (3.3 pg)<sup>7</sup>

The ethanol-precipitated nuclear matrix DNA was resuspended in 10 mM Tris-HCl pH 8.0. Solid CsCl was added to an aliquot of this DNA to a final density of 1.715 g ml<sup>-1</sup>. The DNA was centrifuged at 39,460 r.p.m. for 18 h in a Beckman model E analytical ultracentrifuge. Micrococcus lysodeikticus DNA  $(\rho_{\text{CoCl}} = 1.731 \text{ g ml}^{-1})$  was used as a marker. Control DNA was centrifuged in a second model E cell at the same time as nuclear matrix DNA. The isolation of control DNA was identical to that of matrix DNA except that high salt-treated nuclear matrices from control cells were not digested with DNase I. The results of analytical ultracentrifugation of both nuclear matrix and control DNA are shown in Fig. 2.

The bovine satellite DNAs comprise ~15% of the total genome. Of the total matrix DNA, ~60% is satellite DNA and 40% main-band DNA—this represents a fourfold enrichment of satellite DNA on the nuclear matrix. All four major bovine satellite DNAs seem to be protected from DNase I digestion (Fig. 2). To corroborate further the enrichment of satellite DNA on the nuclear matrix, a second aliquot of the isolated matrix DNA was digested with EcoRI (see Fig. 3). Fragments characteristic of satellites I (1,400 bp) and IV (970, 1,600 and 2,150 bp) were generated by EcoRI digestion (satellites II and III are undigested by this enzyme<sup>9,10</sup>). The relative enrichment of satellites I and IV is also obvious from the reduction in the background DNA due to main-band contamination (Fig. 3, lanes 2, 3). A reported absence of enrichment of satellite sequences on the nuclear matrix11 may be due to differences in the isolation of matrix-associated DNA compared with methods used here.

A primary role of the nuclear matrix is in DNA replication: the matrix is the site at which DNA replication complexes are fixed<sup>6,12</sup>. DNA is replicated as it moves through the stationary complex13. Unlike that of eukaryotes, the bacterial replication complex is attached to the cell membrane 14-16. The nuclear matrices of SV40-infected 3T3 cells are enriched in SV40



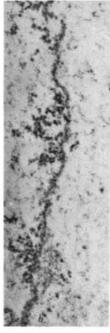


Fig. 1 Electron micrograph of a nuclear matrix from a Madin-Darby bovine kidney cell. Pellets of nuclear matrices were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.3, post-fixed in 1% OsO4 and embedded in plastic. Thin sections were stained with uranyl acetate and lead citrate. a, nuclear matrix appears as thin fibrogranular network. Scale bar, 1 µm. b, Enlargement ( $\times 1.8$ ) of the peripheral region marked (black lines) in a. The periphery appears granular. The absence of a nuclear envelope is due to the use of the detergent Nonidet P-40 in the nuclear isolation procedure.

sequences<sup>17</sup>. This association of viral sequences with the matrix may be due to the recognition and binding of the viral origin of replication by the nuclear matrix. Similarly, the tandemly repeated bovine satellite DNAs18 may contain periodically spaced origins of replication which are recognized and bound by the nuclear matrix.

I conclude that bovine satellite DNAs are enriched on the nuclear matrices of interphase cells. Whether this enrichment is due to the preferential association of nuclear matrices with

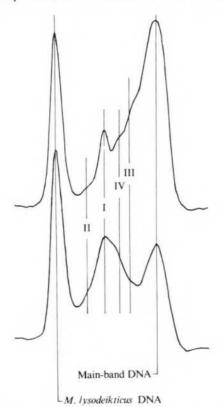


Fig. 2 Analytical ultracentrifugation tracings of control DNA (upper trace) and nuclear matrix DNA (lower trace). The four major density satellites of bovine DNA in CsCl are I = 1.715, II = 1.723, III = 1.705 and IV = 1.710 g ml<sup>-1</sup>. The percentage of the total DNA attributable to satellite or main-band DNA was determined by cutting out and weighing the respective peaks and regions beneath them.

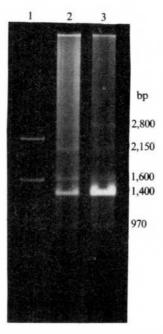


Fig. 3 Gel electrophoresis of EcoRI-digested control DNA (lane 2) and nuclear matrix DNA (lane 3). Electrophoresis was in 1% agarose at 70 V for 5 h in 0.04 M Tris-HCl pH 8.0, 0.02 M sodium acetate, 2 mM EDTA, 0.018 M NaCl. After electrophoresis the gel was stained with 1 µg ml dium bromide, and photo-graphed in UV light. The plasmid pBR322, cut with HincII and AvaI, was added to an aliquot of pBR322 cut with HinfI and used as marker. The EcoRI fragment of satellite I is 1,400 bp; a dimer (2,800 bp) of this satellite is also present. Satellite IV was cut by EcoRI into fragments of 970, 1,600 and 2,150 bp.

origins of replication sequences in satellite DNA or to other satellite DNA sequences is being studied.

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Sixteen contributors discuss the books they particularly enjoyed

#### Paul Davies

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After a decade of reading, consulting and reviewing books on the theory of relativity, I found Sam Lilley's treatment of the subject in *Discovering Relativity for Yourself* refreshing and entertaining, as well as a gold-mine of heuristic devices. The informality of style conjures up the image of a draughty Nissen hut in Skegness, which is, no doubt, where much of the material originated — Lilley is an expert on teaching relativity to carpenters, miners and housewives at Adult Education classes. A curious amalgam, this book, of American self-paced inquiry and quaint, Old World, good-for-the-soul instruction.

#### Glyn Daniel

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We have also been waiting for years (indeed it seems to have been ten years in the making) for the first volume of the Cambridge Agrarian History of England and Wales. Here it is: Vol.I, Part I Prehistory, edited by Stuart Piggott. The editor himself deals with early prehistory, Peter Fowler covers later prehistory — from the Beaker folk to the Romans — and then there is a survey of some 100 pages on the livestock of southern Britain from prehistory to AD 1042 by Dr M.L. Ryder. This book is essential reading for everyone concerned with ancient Britain.

#### **Ashley Montagu**

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#### Eric Ashby

AMONG books published over the year, I select two which are straws in the wind of opinion among biologists. One is Robert Boardman's record of the international movement to conserve nature, *International Organization and the Conservation of Nature*. The other is Rupert Sheldrake's *A New Science of Life*, an astonishing challenge to orthodox theories of plant and animal development.

Boardman provides impressive evidence that tens of millions of people (over three million in Britain alone) are enrolled in a campaign to protect nature from hazards caused by mankind; something inconceivable 50 years ago on this scale. Sheldrake's book recalls the writing on holism 50 years ago by Smuts and Whitehead. But he adds a new dimension: he offers to put his hypothesis to the test of experiment. Boardman confirms our attitude of respect for nature. Sheldrake challenges our respect for reductionism.

#### Stephen Jay Gould

I WILL cheat a bit and delve back a few months into 1980, since I recommend (in any case) a book for all ages. My choice is, of all things, an index — but no ordinary index. It is the last volume of the monumental Dictionary of Scientific Biography, published in 16 parts from 1970 to 1980 with C.C. Gillispie as general editor.

No ordinary, dry accounts of basic facts in the lives of great scientists, these — but intellectual essays (in biographical format) on the social context of science, the nature of creativity and the passion for learning that makes our profession such a joy at its best. Browse and learn.

#### Stuart Sutherland

THIRTY years ago it was customary for experimental psychologists to present new work in book form. The publication of Donald Hebb's The Organization of Behavior in 1949 and of J.J. Gibson's Perception of the Visual World a year later aroused great excitement. Now that psychology has — almost — attained the respectability of a science, those heady days are over. New work appears in journals and most books on the subject merely synthesize existing research.

Of recent psychology books, the two I have most enjoyed are written in non-technical language and can and should be read by workers in all branches of science. In *The Mind's Best Work*, D.N. Perkins summarizes his own research on creativity and by showing that the underlying mental processes are no different from those used in everyday life debunks the mystery of creation that Arthur Koestler and others of his ilk have tried so hard to foster. George Miller's *Language and Speech* presents a lively summary of all aspects of work on language. It has the rare defect of being too short and leaves the reader avid for more.

#### **Nevill Mott**

In James Clerk Maxwell: A Biography, Ivan Tolstoy declares that his subject should be ranked next to Newton and Einstein. He sets out to explain why to the non-scientific reader, who, he says, may well have never heard of Maxwell. Of course, Maxwell's greatest achievement was his equations for the electromagnetic field, and according to the author what was epoch-making in this was the replacement of Newton's action at a distance by the concept of an electromagnetic field.

On reading this book, I enjoyed being reminded of my first acquaintance with Maxwell's equations as an undergraduate, and my feeling that the concept of the displacement current was a supreme example of the power of mathematics to show that what is, must be. I can think of only one comparable example, Dirac's proof that an electron which obeys the laws of relativity and of quantum mechanics must have a fourth degree of freedom. Such things are at the very summit of theoretical physics, and I much enjoyed Professor Tolstoy's attempt to bring this great man's life before

#### **Lewis Thomas**

ON A complicated planet, meaninglessness keeps popping up as explanation: the world makes no sense; we are alone in jumpy randomness, getting nowhere. Amidst this confusion, 1981 provided us with three guide books.

In Laws of the Game, Manfred Eigen and Ruthild Winkler glimpse, at a distance, the world at play. Genetic rearrangement, generative grammar and Bach take the same chances, live by the same rules.

Donald Griffin's *The Question of Animal Awareness* is out to show that quick thinking, not all that different from what we think is thinking, abounds. A bee knows what bees are up to. Bats puzzle their way through the dark. Tiny brains can have tiny thoughts.

With high zest, in Symbiosis in Cell Evolution Lynn Margulis presents the case for cooperative living as a triumph of evolutionary fitness, an intricate but sure way up life's ladder, the best of games.

#### William T. Golden

Would'st thou hear what Man can say In a little? Reader, stay.\*

Statistical Abstract of the United States (101st Annual Edition) presents extensive information on the country's social, political and economic organization. Informative texts introduce the 34 sections (Agriculture, Education, Population, Science, for example); 1,616 tables, 71 charts in 1,059 pages. Something for everyone.

D'ye ken frog from toad, street from road, seer from prophet, amulet from talisman, wasp from hornet; and hundreds more? Then, for fun and edification consult Room's *Dictionary of Distinguishables*.

Leonardo da Vinci: the complex genius, peerless artist, technological visionary, military inventor and opportunist, stirs mind and soul in Martin Kemp's cogently written, generously illustrated analysis. Truly the marvellous works of nature and man.

\*Ben Jonson, Epitaph on Elizabeth L.H.

#### June Goodfield

STEPHEN Jay Gould's *The Mismeasure of Man* achieves several things, one of which is outstanding. By superlative historical scholarship and critical analysis, he demolishes those attempts to limit the lives of other human beings by "measuring" their intelligence whether by the dimensions of their skulls — as in the nineteenth century — or by testing their "supposed" IQs in the twentieth.

His achievement is, first, to demonstrate the strengths and limits of a quantitative measure in science; second, to show that even while science truly *cannot* ever be wholly detached and objective, for prejudices and unconscious attitudes do indeed influence what its practitioners see and interpret, the enterprise nonetheless *does* provide us with a marvellous method for challenging the *status quo*, as well as revealing firm knowledge about the world; third, he reaffirms how much is humanly possible.

#### J.Z. Young

RICHARD Leakey's *The Making of Mankind* provides an objective and also beautiful summary of the known evidence about human ancestry. He deals with the facts of the evolution of culture, language and even of civilization and cities, as well as of skulls. He concludes with some wise thoughts about the present, in which he rejects the views of Ardrey, Lorenz and others that human beings are unredeemably aggressive.

This book is a fitting sequel to Solly Zuckerman's classic, *The Social Life of Monkeys and Apes*. When first published, in 1932, it provided many new insights into primate and human reproduction. Now it has been re-issued with a further contribution from the author, who also includes some sensible thoughts about the future.

#### A. Hallam

THERE have recently been some exciting developments in palaeontology. For lay readers, especially biologists, who want a clear, non-technical but authoritative account of the study of fossils from an ecological, biogeographical and evolutionary point of view, written in a lively style, 1981 brought Chris Paul's *The Natural History of Fossils*.

For specialists, the textbook by J.R. Dodd and R.J. Stanton, *Paleoecology: Concepts and Applications*, is a sound, comprehensive and well-documented review of recent research — it may well become the standard reference work for some years to come.

#### L. Beverly Halstead

I was delighted to find in Michael Ruse's new book, Is Science Sexist?, a philosopher stoutly defending evolution. Neo-Darwinism is under attack from many directions. The anti-Darwinians seem to be recruiting new forces: including two kamikaze pilots proclaiming Evolution from Space directed by a cosmic intelligence from South Wales. At this most critical juncture, we find a new vigorous army, philosophical under Michael Ruse, sweeping across the landscape scattering all before it. I know exactly how Wellington must have felt at Waterloo when Field Marshal G.L. von Blücher and his Prussian troops came into view. The war is not yet over but the final outcome is now assured. Charles Darwin would have been pleased to know you Michael Ruse.

#### Isaac Asimov

THE book that pleased and edified me most in 1981 was *Science: Good, Bad and Bogus* by Martin Gardner.

The writing is not strictly that of 1981, for it is a collection of Gardner's essays on various forms of pseudo-science that date back over the past 20 years. Still, aside from the sheer enjoyment Gardner's clear, rational and occasionally sardonic style brings me, 1981 is a year in which his sane voice is enormously important.

Pseudo-science and non-science is on the rise in the United States and the self-named "moral majority" is bringing the force of obscurantist religion into the fight against freedom of thought. Men like Gardner must make their voices heard and in this book he does so most effectively.

#### P.B. Medawar

THREE books have struck me greatly during the year. First, C.P. Snow's enthralling *The Physicists*, with an all-star cast. Olympian figures are not necessarily the most interesting or instructive but Snow makes them so because he is big enough himself to see them to scale. Snow himself, shrewd, learned and magnanimous, comes out as well as anyone.

J.M. Tanner's A History of the Study of Human Growth is notable as a history of important ideas — seen in a context of intellectual and social history.

Finally, M.F.A. Woodruff's *The Interaction of Cancer and Host: Its Therapeutic Significance* is a *tour de force* with the breadth and depth of a multi-author volume, yet the work of a single first-rate mind

#### **Eugene Garfield**

I MET V.V. Nalimov, the well-known Soviet statistician and cybernetician, at the first Moscow Book Fair. We were brought together by our mutual, long-standing interest in scientometrics. After I read his manuscripts, I recommended that we should publish two of his books.

In the Labyrinths of Language: A Mathematician's Journey investigates the languages of biology, art, mathematics, statistics and philosophy. Nalimov proposes a taxonomy of languages, which describes the spectrum-continuum of language, from the "hardest" (mathematics) to the "softest" (art, Hindu metaphysics).

Nalimov ranges even more widely in Faces of Science. In these essays, each reflecting a different aspect of science, he demonstrates that science is a large, self-organized information system. How could I, as a linguistics-trained, essay-writing, information scientist, resist these fascinating ideas?

# Details of publishers and prices

Agrarian History of England and Wales. Vol. I, Part I Prehistory. (Cambridge University Press.) £27.50, \$64.50.

Dictionary of Distinguishables. (Routledge & Kegan Paul.) \$12.95, £5.95.

Dictionary of Scientific Biography. (Charles Scribner's Sons.) 16 volumes, \$595. Single volume concise edition \$100.

Discovering Relativity for Yourself. (Cambridge University Press.) Hbk £17.50, \$49.50; pbk £7.95, \$19.95.

Evolution from Space. (Dent.) £7.95.

Faces of Science. (ISI Press.) \$22.50 US, \$25.50 elsewhere.

The Heyday of Natural History. (Cape/ Doubleday.) £9.50, \$17.95.

A History of the Study of Human Growth. (Cambridge University Press.) £30, \$69.

In the Labyrinths of Language: A Mathematician's Journey. (ISI Press.) \$22.50 US, \$25.50 elsewhere.

The Interaction of Cancer and Host: Its Therapeutic Significance. (Grune & Stratton/Academic.) \$46.50, £26.20.

International Organization and the Conservation of Nature. (Macmillan Press.) £15.

Is Science Sexist? (Reidel.) Hbk Dfl.80, \$42; pbk Dfl.32.50, \$14.95.

James Clerk Maxwell: A Biography. (Canongate.) £9.95.

Language and Speech, (W.H. Freeman.) Hbk £8.20; pbk £3.50.

Laws of the Game. (Knopf.) \$19.95.

Leonardo da Vinci: The Marvellous Works of Nature and Man. (Harvard University Press/Dent.) \$30, £14.95.

The Making of Mankind. (Michael Joseph/ Dutton.) £9.95, \$24.95.

The Megalithic Art of Western Europe. (Clarendon/Oxford University Press.) £50, \$85.

The Mind's Best Work. (Harvard University Press.) \$18.50, £12.95.

The Mismeasure of Man. (W.W. Norton.) \$14.95.

The Natural History of Fossils. (Weidenfeld & Nicolson/Holmes and Meier.) Hbk £15, \$37.50; pbk £6.95.

A New Science of Life. (Blond & Briggs/ Tarcher.) £12.50, \$11.95.

On Becoming Human. (Cambridge University Press.) Hbk £20, \$29.95; pbk £6.95, \$10.95.

Paleoecology: Concepts and Applications. (Wiley.) £29.55, \$53.15.

The Physicists. (Macmillan Press/Little, Brown.) £8.95, \$15.95.

The Printing Press as an Agent of Change. (Cambridge University Press.) Pbk £12.50, \$16.95.

The Question of Animal Awareness. (Rockefeller University Press.) \$13.95.

Science: Good, Bad and Bogus. (Prometheus.) \$18.95.

The Social Life of Monkeys and Apes, 2nd Edn. (Routledge & Kegan Paul.) £17.50, \$55.

Statistical Abstract of the United States (101st Annual Edition). (US Government Printing Office.) Hbk \$14, pbk \$11.

Symbiosis in Cell Evolution, (W.H. Freeman.) Hbk \$24.50, £16.40; pbk \$14.95, £9.20.

## Archaeoastronomical anomalies

Clive Ruggles

The First Stargazers: An Introduction to the Origins of Astronomy. By James Cornell. Pp.262. ISBN US 0-684-16799-9; ISBN UK 0-485-30004-4. (Charles Scribner's Sons/Athlone: 1981.) £7.95, \$15.95.

ARCHAEOASTRONOMY is a new and potentially exciting discipline. Its precise aims - broadly, investigation of the astronomical practices of ancient societies through various aspects of their material legacy - and limitations are not (yet) well defined; it includes on the one hand studies of Mesoamerican structures, where site evidence can be considered alongside written evidence from codices and ethnohistoric evidence, and on the other studies of British megalithic sites, where conclusions depend almost entirely upon the statistics of alignments and horizon indications. Almost all workers in the field are part-time, retired or amateur, and their main interests cover a variety of academic disciplines. The scientific value of much archaeoastronomical evidence has still to be properly determined; methodological improvements are only now generally being made. Thus the status of much potentially interesting field work has yet to be reassessed and confirmed.

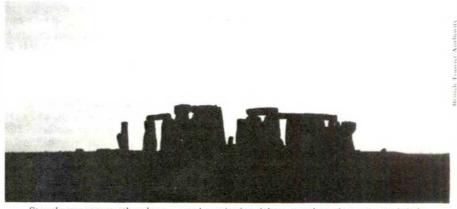
All of this presents the popular writer on archaeoastronomy with a number of problems. First, he has to cope with a confusing diversity of material, geographically and in terms of approach and quality. Second, he has to give some account of the difference between testable hypotheses and valueless speculation, and distinguish between different theories on these grounds. Finally, he has to combat the many popular misunderstandings, and be careful not to be drawn into endorsing purely speculative or now-refuted ideas on the grounds that they provide exciting material and help to sell books.

On the first count James Cornell has coped very well indeed; material is organized into chapters on a geographical basis, and general points are made whilst discussing particular sites or theories.

However on the second and third counts the book is a mixed bag, indeed: on occasions the author has done an excellent job, but on others he has failed dismally, and, worse, will badly mislead.

There are many instances in the book where valid theory is well separated from mere speculation, and the point is often made that deliberate astronomical orientations need to be distinguished from mere chance occurrences. In the opening descriptions of equinoctial sunset over a pyramid at Chichén-Itzá in Mexico, Cornell follows a description of a shadow phenomenon by asking "is this merely coincidence?" and explaining why he thinks it is not. Later he uses the example of a rock carving at Toro Muerto in Peru to illustrate a point about subjectivity and different possible interpretations. In the chapter on Egyptian temples, a reasonable approach (by Gerald Hawkins) is well distinguished from the speculations of various "pyramidologists". A good account is given of both sides of an argument about the astronomical interpretation of rock art. The "Sirius mystery" of the Dogon tribe in Africa, and its probable explanation in terms of earlier contact with someone from the outside world, is entertainingly recounted. And so on.

The author is not, however, always so clear about why he is ready to endorse some theories and dismiss others, and does not always identify the more speculative theories as such. Thus we hear that Alexander Marshack's ideas on lunar notation amongst bone scratches from the Cro-Magnon period are "steadily winning support throughout the scientific community". There is scarce mention of a more objective approach, which might ask how many other interpretations could equally well have been placed upon the bone marks. Instead, we are told how a "background as a journalist, screenwriter and popularizer of science does not help win support in the scientific community". The author might reflect that in some cases this might be due to an inadequate apprec-



Stonehenge sees another dawn — and continuing debate over how the stones and their alignments should be interpreted.

iation of the rigours of the scientific method.

Mr Cornell is at his most dogmatic in the chapter on Stonehenge, which is disappointing and, indeed, infuriating. Here we see regurgitated the Stonehenge debate of the 1960s, with Gerald Hawkins hailed as "having refuted nearly a century of archaeological dogma" and "having begun a revolution that would lead to a complete rethinking of the intellectual development of humankind", while archaeologists such as Richard Atkinson are denigrated for daring to criticize, and are said to be motivated by their "traditional view of these people as backward barbarians". In the same chapter Lockyer's earlier theories are discussed and justly criticized, but when it comes to Hawkins there is suddenly a flagrant disregard for any objective discussion. Instead, as one encounters more and more references throughout the book to Hawkins's "pioneering research at Stonehenge", how he has been praised as the "father of archaeoastronomy", and how he "almost single-handedly changed public and scientific conceptions of archaeoastronomy", one begins to wonder if this isn't really a Gerald Hawkins tribute

It is certainly true that Hawkins's work on Stonehenge led to a general public interest in archaeoastronomy and it is undeniable that Hawkins has done much work of value in this field, but the work on Stonehenge itself was soon criticized on justifiable grounds. It is perhaps worth (tiresome though it is to have to do so, for it has been done several times) briefly putting the record straight here. The astronomical interpretation of Stonehenge I rests on the finding that 24 out of 50 possible alignments between pairs of features at Stonehenge I were of astronomical significance. The chances of this happening fortuitously were calculated by Hawkins to be less than one in a million, as quoted by Cornell. In fact, there were 24 lines out of a total possible of over 100, since alignments both ways along most pairs were allowed as possibly astronomical; Hawkins calculated the probability of exactly 24 astronomical "hits" rather than 24 or more, and different tolerances were allowed on different lines. When these errors are taken into account, no evidence whatsoever remains for preferential astronomical orientation. This is quite apart from any archaeological and other arguments about the choice of features for inclusion in the analysis. These errors are simple ones certainly not a case for "mathematicians still arguing" as stated by Cornell - and, although they were pointed out by Atkinson only a year after the publication of Hawkins's Stonehenge Decoded (Doubleday, 1965), even now they are still present in current reprints of the book. This fact does not imply any great commitment to objective research, and it is tragic that Cornell's book, if widely read,

will only revitalize public misconceptions on this point. Cornell rightly points out that Hawkins's conclusions "never seemed to bother a technically attuned public": with the exception, one may surmise, of those with a questioning mind and some high-school mathematics.

In the chapter on Stonehenge and British archaeoastronomy in general, the author dismisses the archaeoastronomical work of Professor Alexander Thom in less than three pages, and mentions later that Thom's work scores a very low "reliability value" according to criteria devised by Hawkins (under which, not surprisingly, Hawkins's own work scores a high value). This sort of denigration is insidious. Thom has investigated a large number of sites, presented his results in a manner so that they can be readily re-examined and criticized and has been prepared to modify his opinions in the light of criticism and new evidence. (I am amongst those attempting critical assessments of his work.) In this manner, hypotheses are presented, criticized and modified, a procedure in accord with the scientific method but perhaps less susceptible to large-scale popular interest.

There are a number of minor errors in

the book which there is no room to mention in detail, particularly amongst the astronomical background material cunningly concealed amidst discussion of Toro Muerto and Marshack's work (that it is cunningly concealed is not a criticism).

But the overriding criticism of this book must be its one-sided and misleading treatment of British archaeoastronomy, in particular the almost fanatical praise of Hawkins and detriment of his critics. The complete break with objectivity in this respect must invalidate the value of the rest of the book, at least for non-specialist readers (and this is the intended audience) who cannot judge for themselves. Indeed, it will quite possibly have a worse effect, for in raking up past confusions and prejudices, amongst an objective discussion of other fields of archaeoastronomy, together with its several references to the prejudice of archaeologists against new ideas, it threatens to direct popular support towards sensationalism and away from level-headed scientific research.

Clive Ruggles is University of Wales Research Fellow in the Department of Archaeology at University College, Cardiff.

### **Exploring Britain's prehistoric past**

Ruth D. Whitehouse

The Penguin Guide to Prehistoric England and Wales. By James Dyer. Pp. 384. ISBN 0-7139-1149-6. (Allen Lane: 1981.) £9.50.

POPULAR interest in archaeology in the British Isles has never been higher, an interest which has brought increasing numbers of visitors to the visible remains of our past, our ancient monuments. James Dyer's book is a concise guide to almost a thousand of the most interesting sites of the prehistoric period in England and Wales. It is not the only guide of its kind: competitors include Nicholas Thomas's Guide to Prehistoric England (Batsford, 2nd Edn 1973), Richard Wainwright's A Guide to the Prehistoric Remains in Britain: South and East (Constable, 1978) and James Dyer's own Southern England: An Archaeological Guide (Faber, 1973).

The advantage of the new Penguin guide over these other works is its wider geographical coverage and the larger number of sites included. It begins with a section of five maps and a short introduction to British prehistory. The main part of the book consists of a gazetteer of sites, listed alphabetically by county. The gazetteer itself is excellent: the accounts of sites are concise and accurate, with Ordnance Survey grid references included; many of the site descriptions include one or more bibliographical

references and some are illustrated with photographs or line drawings. The visitor is therefore provided both with clear descriptions and an introduction to further reading.

Less satisfactory is the introduction. Archaeology today is developing fast and is characterized by controversy and debate on almost every topic. Dyer ignores this and presents a consensus view of British prehistory, which may accurately reflect majority opinion, but which neglects the new approaches and hypotheses that have transformed the subject in recent years. To take a single example, the Beaker pottery and associated artefacts of the third millennium BC are explained in terms of people migrating into and across England. This is indeed the traditional interpretation, but there is an alternative view that they represent a "status kit" of prestigious objects which were acquired by influential and wealthy people in different societies and were distributed not by migration, but by trade or exchange. While a guide book is not perhaps the proper forum for the airing of controversy, the onus surely remains to provide an up-to-date level of interpretation. Nonetheless, this is a book that everyone interested in Britain's prehistoric past will wish to own.

Ruth D. Whitehouse is a Lecturer in Prehistoric Archaeology at the University of Lancaster.

#### Odds and ends about the dead and buried

Stuart Piggott

Rites of the Gods. By Aubrey Burl. Pp. 258. ISBN 0-460-04313-7. (Dent: 1981.) £12.

WITH its meretricious title and dust-jacket, is Mr Burl's book going to lead us to take the "misdirected step" that "leads one towards apparitions, White Goddesses, psychic archaeology and the Never-Never land of the ley-liners"? Of course not: the deservedly caustic phrases are those of the author. But his book is not quite what it might seem to be, for it is not a discussion of ancient non-Christian cults, not even of those inferred for prehistoric Europe, but is limited in space to the British Isles and in time to a period c. 4500-1000 BC, with sketchy chapters for the phases before and after. Within these limitations Mr Burl presents to the non-archaeological reader some of the inferences which can be made as to ritual practice and performance from the material culture of burial and ceremonial monuments, a field to which he has made important contributions.

In the six chapters which form the bulk of the book the treatment is not thematic but topographical, with what seems a capriciously selected series of field monuments, some surviving, some destroyed or invisible from the ground. This does not make for easy or even very interesting reading, and obscures the real topics which could have been explored, such as the variations perceptible in the rites of collective burial - successive, redeposited, partial or complete - or a fuller treatment of the claims for astronomical expertise in British prehistory, on which the author has made pertinent comments elsewhere that deserve re-statement. Unfortunately, too, there are many errors of detail in the site descriptions, and the reports of early antiquarian diggings are sometimes given more credence in detail than they deserve. Modern "folklore" is also dealt with uncritically and without regard to the absorption by popular tradition, from the Renaissance onwards, of literate antiquarian speculations. Even less sure is the handling of anthropological evidence, where a sprinkling of words such as "shaman", "totem", "mana" or "sympathetic magic" hardly inspires confidence, and Ucko's classic paper of 1969 (World Archaeology I.2, 262-280) on precisely the problems posed by the interpretation of prehistoric funerary practice is not mentioned, though it should be required reading for all concerned. The limitations of inference from "grave archaeology" are nowhere set out.

Surprisingly, when in the later first millennium BC something, however tenuous, becomes explicit in Celtic religion, the book tails away. Here it is strange, in view of the prominence rightly given to henge monuments and allied structures earlier in the book, that nothing

is said of the evidence discussed in several recent papers for an insular survival of this pre-Celtic sanctuary tradition in such sites as Dun Ailinne or Emain Macha (and Tara), with probable British counterparts; the circular timber Iron Age temple beneath the Roman structure on Hayling Island is among other new and important cult sites of the period.

All in all, Rites of the Gods cannot be called a success, and does little more than present the reader with a scrap-book of miscellaneous data, too often interpreted in terms of modern modes of thinking. I had expected, and the general public deserves, something much better from Mr Burl

Stuart Piggott retired as Abercromby Professor of Archaeology at the University of Edinburgh in 1977. His books include Ancient Europe (Edinburgh University Press, 1965) and The Druids (Thames & Hudson/Pelican, 1974).

### Jupiter and the Moon displayed

David W. Hughes

I FIND map books fascinating and spend many happy hours investigating a specific country and tracing the lines of equal height, geological structure, nationality, temperature, rainfall, population, magnetic inclination and so on. The fascination seems to be a function of the amount of information displayed, so turning to the planets the first question is, "Do we know enough to fill a good atlas?". Looking at the two atlases under review one also must ask if all the relevant information has been included.

The first two volumes in The Mitchell Beazley Library of Astronomical Atlases for Amateur and Professional Observers deal with Jupiter and the Moon. Jupiter has been written by Garry Hunt and Patrick Moore, who divide their attention equally between the planet and its satellites. There is no doubt about there being enough knowledge to fill an atlas here. The images from Voyager 1 and 2 and Pioneer 10 and 11, apart from revealing three new satellites and a ring around the planet, have provided a host of superb pictures. This atlas reproduces many of these in colour. It not only provides maps of Jupiter and its satellites, but it also reviews our knowledge of such topics as The Great Red Spot, the interior of Jupiter, the magnetosphere, the effect of Io on Jovian radio emission, cloud morphology and colouring, volcanism on Io, surface fracturing on Europa, and cratering on Callisto and Ganymede.

The book is well written, physical and astronomical terms being clearly and correctly defined.

After the excitement of the Pioneer and Voyager fly-bys we are now scientifically becalmed and must return to "oldfashioned" visual and photographic observations from Earth. No Earth-based telescope can show anything like the amount of detail provided by space imaging, but Jupiter can be kept under observation for a large fraction of each year. It is thus well worth mentioning that the bible of Jupiter watchers - Bertrand M. Peek's The Planet Jupiter, The Observer's Handbook - has been reissued in a revised edition (Faber and Faber; £10, \$29.95). Needless to say, with 170 pages devoted to observations of the belts and zones of Jupiter and their temporal variation, this book is not for the faint hearted.

The second atlas, *The Moon* by Patrick Moore, reflects, to quote the author, "the endless enjoyment to be gained simply from looking at the Moon". Despite being visited by astronauts and a multitude of unmanned probes, the Moon has lost nothing of its romance. Again, the atlas contains about 16 pages of colour plates and a large collection of stereographic, orthographic and Mercator maps of lunar regions.



The mobile man in the Moon — James B. Irwin and the Lunar Roving Vehicle. The picture is taken from Joseph Jackson and John Baumert's *Pictorial Guide to the Planets*, 3rd Edn, just published by Harper & Row, price £9.50.

Moore provides a basic introduction to crater morphology, lunar chronology, mascons, transient lunar phenomena, regolith formation and the recent space exploration programmes. I would have liked to have seen one or two coloured geological maps (a typical example could have been the US Geological Survey map of the Copernicus quadrangle) and maybe a detailed contour map would have been fun (NASA's lunar topographic orthophotomap of the Apollo 15 Hadley region would do). Additional detail about surface temperature variation, transient atmospheres and the present meteorite influx rate would also not have been amiss.

We all have our favourite crater and I was surprised that mine, Giordano Bruno (which *might* have been formed as recently as July 18, 1178), doesn't even rate a mention. However Moore has produced an ideal book for the amateur observer, and anyone who ever fancies turning a telescope or a pair of binoculars onto the Moon will find this atlas an excellent starting point and guide.

Despite the grandiose title given to the series by Mitchell Beazley I think the two atlases, especially *The Moon*, fall well into the amateur division of the astronomy book league. I must say, though, that at £6.95 each (in the US \$14.95, Rand McNally) their value for money will be hard to beat.

David W. Hughes is a Lecturer in Physics and Astronomy at The University of Sheffield.

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### Guidance for the observant amateur

Patrick Moore

A Complete Manual of Amateur Astronomy. By P. Clay Sherrod. Pp. 319. ISBN hbk 0-13-162115-7; ISBN pbk 0-13-162107-6. (Prentice-Hall: 1981.) Hbk \$24.95, £18.70; pbk \$10.95, £8.20. The Practical Astronomer. By Colin A. Ronan. Pp.153. ISBN UK 0-333-31245-7; ISBN US 0-02-604500-1. (Macmillan, London/Macmillan, New York: 1981.) £8.95, \$20.

ALTHOUGH no book can be really regarded as complete, Clay Sherrod's *Manual* is certainly exhaustive, and it contains much up-to-date information which cannot be found elsewhere in convenient form. The author is a well-known amateur astronomer, and his experience will be found to be very useful to others.

Topics covered range from telescope selection and maintenance to naked-eye work (meteor observation, for example); studies of the Moon and planets; photoelectric photometry, and all kinds of astronomical photography. The sections dealing with equipment are of special value. Many amateurs tend to believe that setting up a photoelectric photometer is too much of an undertaking; reading this book may cause a change of outlook. The author stresses, rightly so, that the modern amateur has to be much more specialized than his predecessor of half a century ago.

There are, unfortunately, some blemishes, and the weakest parts of the book are those dealing with the Moon and planets. In the lunar section, no mention is made of transient lunar phenomena, even though it is here that the amateur can carry out his most valuable work. In some ways the information given is out of date: for instance, it is no longer believed that lunar rills are "dried beds of water flow" - this idea was abandoned many years ago, and analyses of the lunar samples brought home by the Apollo astronauts and the Russian Luna probes have shown that there are no hydrated materials on the Moon. The map of Mars given on p.141 is, frankly, of no use, and the list of Martian features includes many canals and other markings which do not exist at all - so that the amateur will be hard pressed to locate them! The most surprising statement is that in Saturn's system, "the satellites Titan, Mimas and Hyperion are those that you should study most during transit events". Any observer using a modest telescope to watch a transit of Mimas or Hyperion would indeed have to have keen vision.

There are comparable mistakes in the stellar lists. On p.204, it is said that Antares varies from magnitude 0.9 to 1.8 — in fact its fluctuations are too slight to be detected without using a photometer or some such device; Alpha Cassiopeiae never rises to magnitude 1.4, and even its variability is questionable (Kukarkin's catalogue omits it); presumably n Aquilae should be

 $\eta$  Aquilae, R Geminorum should be U Geminorum, and so on. These errors are trivial in themselves, but in a book aimed at providing a guide for amateurs they can be highly misleading.

All in all, it seems that the proof-reading has been inadequate, and that the author is not fully conversant with some of the latest research. But the merits of the book far outweigh the defects, and there is nothing which cannot be put right in the next edition. When this has been done, the book may well become a standard work.

Colin Ronan's book, The Practical Astronomer, is much more elementary, and aimed at the younger reader. As well as providing a general introduction to astronomy, it contains many practical demonstrations and experiments; the text is clear and riveting, and the illustrations excellent. A book to be strongly recommended.

Patrick Moore is an author and astronomer. His recent books include The Moon, Jupiter (with Garry Hunt) and The Atlas of the Universe, all published by Mitchell Beazley.

#### Cosmic methadone

William H. Press

The Edge of Infinity: Naked Singularities and the Destruction of Spacetime. By Paul Davies. Pp.194. ISBN UK 0-460-04490-7; ISBN US 0-671-44063-2. (Dent/Simon & Schuster: 1981.) £7.95, \$14.50.

"BEYOND black holes to the end of the universe", trumpets the cover blurb. The author, with more modest affect, relates a conversation among colleagues bemused at the plethora of black-hole books: "'Whatever next?' someone asked. 'The naked singularity!' came a prompt reply. So I have written, with some amusement, a book about naked singularities".

A naked singularity, in modern relativity theory, is a region of spacetime where gravitational forces become infinite, crushing matter to infinite density, but from which light signals can escape, rendering the singularity visible. The singularity inside a black hole is not "naked" the choice of this word is usually attributed to Roger Penrose - but is, rather, modestly clothed behind an opaque "event horizon" (the surface of the black hole) through which outward light signals cannot propagate. The big-bang singularity from which our Universe expanded is, however, naked: the 3K cosmic microwave background is a signal from that singularity (or, at least, from as close to that event as the opacity of dense, ionized matter will allow us to look).

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In claiming to have given us a book "beyond black holes", the author is gently pulling our legs. Really, the book is another black-hole book — quite a fine specimen of the genre — and it is also about all those other popular buzzwords which have leaped from relativity theory into the public domain: warped spacetime, gravitational collapse, the big bang, Schwarzschild radius, Hawking radiation and so on

The market for these books seems as insatiable as that for Rubik's cubes. It is out of this crowded pack that Paul Davies has emerged as a prolific and, I think, major figure in popular science writing. This book is his sixth or so in less than five years. They keep getting better and better. Davies writes with a clear, sure, sane voice, at once understandable and astonishingly close to the Nirvana of total technical accuracy.

There are other good, popular blackhole books of course. One, Iain Nicolson's Gravity, Black Holes, and the Universe, was praised by Paul Davies recently in Nature (290, 656) in phrases even more applicable to Davies's own book: . . . maintains a reliable scientific perspective and a careful treatment, yet covers most of the 'fun' topics in detail". What sets Davies's book apart is not just that it is good, but that it is determinably good, written by an author with a nowestablished corpus of good science writing behind him and (one hopes) with more still to come. Librarians can confidently put him on their automatic-purchase lists.

Who are Davies's readers? One understands, to be sure, that there are two entirely distinct audiences for popular science, each blissfully ignorant of the other's existence. Group A, beloved by "serious" science popularizers, is imagined to consist entirely of educated country gentlemen and intelligent highschool students. Group B, the targets of science writers who want to make some money, are the gee-whizz junkies, uncritically addicted to the wildest and



Paul Davies — "a clear, sure, sane voice" for both country gentlemen and the gee-whizz junkies.

most irresponsible sorts of speculation. Astonishingly, I think that Paul Davies, in this book, will appeal to both groups. He is onto something that is either a great scam, or a great advance, in science popularization: the Group B addict is lured by a flashy pitch, promised the excitement that he so badly needs. What he gets instead is a

sane and accurate exposition, a detoxification instead of a "high". The Group A reader, meanwhile, not only finds the pitch amusing, but also the actual content entirely satisfactory.

William H. Press is Professor of Astronomy and of Physics at Harvard University.

# A new dimension to evolutionary theory?

D.A.J. Tyrrell

Evolution from Space. By Fred Hoyle and Chandra Wickramasinghe. Pp.176. ISBN 0-460-04535-0. (Dent: 1981.) £7.95.

THIS is in many ways an attractive book. The cover suggests the sky at night, but it is actually a photograph of "cell bodies in mollusc" culled from Nature — a clever visual linking of the ideas in the book. And the book is clever too; it ranges with relaxed ease over some biological oddities, such as the fact that Drosophila responds to UV light of wavelengths too short to be found on Earth, that Vicia faba, a member of the pea family, makes haemoglobin and that some bacteria are incredibly resistant to radiation. In style it seems intended for the general reader, and expounds a grand idea rather than presenting a closely argued case.

The idea is that biological evolution is widely regarded as proved and a complete explanation for the living world as we see it — yet there are awkward facts such as those I have just mentioned which to Hoyle and Wickramasinghe show that genes borne on particles in space have been incorporated into organisms on Earth. They also think this explains the sudden appearance of new species and the existence of gaps in the fossil record. They used essentially the same idea to "explain" in their earlier writings what they regarded as inexplicable features of infectious diseases; then the explanation was germs from space.

The authors make their own low estimates of the frequency of certain mutations and of the improbability that the amino acid sequence of any biologically significant protein could arise "by chance" in a primaeval soup. They then resolve the dilemma by mentioning Arrhenius' theory of "panspermia" and reformulating it by saying that "biomaterial" is parked on particles in space protected by low temperature and low gas concentration, and defended by graphite from radiation. They argue that the material on particles in a certain size range would remain viable after impinging on Earth, as described in their earlier books. In Chapter 7 this is likened to sprinkling new "computer programs" into computers in the planetary system and beyond - that is, new genes into species living throughout the Universe. They point out that studies which apparently showed

unexpectedly large numbers of bacteria in the upper air were discounted by NASA as due to contamination and were not followed up.

I think I understand the dilemma which made Hoyle and Wickramasinghe write the book. The intellectual difficulties of conceiving how life began and developed are stupendous, and the facile suggestions they sometimes include make one doubt the plausibility of some of the proposed schemes — in my own field I cannot see viruses as the "original" life since all those we know need energy and translation systems taken from whole cells in order to reproduce.

So, we agree that is difficult to explain how the first life forms were put together and why the fossil record suggests that either there were no links between certain species or evolution went very fast on a geological time scale. But this book merely moves these problems without solving them - for how were the genes in space created in the first place? And how, once landed on the Earth, were they incorporated into the genome of species already here? Genetic engineers can create environments in which genes can be implanted from without but the likelihood of this happening with extraterrestrial material in a natural environment is to me, intuitively, much less than that of evolution from time to time going so fast in so few animals that no fossil record has been found.

The authors are apparently sympathetic to the idea of a creative God, though most of the references are to one or more "intelligences", designated as ?, ?? and so on. I get the impression, for many important parts of their structure are not defined, that although they refer to the old theory of whole planets being disrupted and sending their germ plasm into orbit, the authors lean towards a concept of biomaterial being formed, perhaps de novo, in many parts of the Universe - a sort of biological continuous creation. But that again is moving the problem about in time, not solving it. I am afraid their hypothesis reminds me of the theological view labelled as "The God of the Gaps"; in fact they admit that their views are a form of the theory of special creation. Such theories bring in a God when the intellectual going gets tough and manage without between

times. It seems to me that it is very likely that the Universe is the creation of a God, but in that case I take the view that the whole grand structure, the bits I think I understand, as well as the bits I certainly don't, are and always have been manifestations of the same intelligence and creating and sustaining power.

On the same basis, although a Creator obviously could at any time or place suspend the "laws of nature" as we have observed them, we can use the method and approach of science only if we assume that these laws operate consistently in time and space. Here the argument echoes that of the "creationists" and "evolutionists" in the USA. It might happen that a scientific examination forced the conclusion that, apart from the existence of the Universe in time and space, some other specific creation events or insertions of genes from space could be demonstrated to have taken place. Hoyle and Wickramasinghe have not convinced me - though my mind is still open.

D.A.J. Tyrrell is Deputy Director of the MRC Clinical Research Centre, Harrow, Middlesex.

# To be an astronomer

Stephen P. Maran

In Quest of Telescopes. By Martin Cohen. Pp.131. ISBN 0-9333-4625-5. (Sky Publishing, Cambridge, Mass: 1981.) \$11.95.

MARTIN Cohen, an expert on T Tauri stars and cometary nebulae, and an English astronomer in the grand tradition of observation and discovery, has written his "astronomical autobiography". Unashamedly still an amateur at heart, Cohen is one of the diminishing breed of professional astronomers who actually enjoy the view through the eyepiece while they make the arcane measurements of the trade.

The book describes a life-long love of telescopes and star-gazing by way of vignettes drawn mostly from time spent at the major observatories of the American West. A photograph of the awed author, dwarfed by the 200-inch Hale reflector, is symbolic representation of the principal theme, a paean to the beauty both of the stars and of the great instruments with which we study them. Helicopter rides in the snow-bound White Mountains aside, his travels to pursue observational research at remote sites in North and South America are typical of those of many astronomers of the present generation who, as Cohen says, "have become jet-set commuters"

Although I began ten years before Cohen and on the opposite shore of the Atlantic, I see that we had many corresponding experiences. The young Martin cycled around town to his first small telescopes and astronomy club meetings; I rode the New York subway to do the same. He twice mistook an internal reflection for a new comet; I watched a student repeatedly "adjust focus" of a Kitt Peak telescope by tweaking what was in fact the counterweight balance adjustment. We both erected mid-sized telescopes in the mountains near Tucson, and apparently both of us found that large, lightweight aluminium mirrors don't work, regardless of what experts once wrote.

In Ouest of Telescopes aims to show high-school students and others what the astronomer's life is like. This is accomplished as much by the brief accounts of how major insights and discoveries were achieved as in the tales of events in Minnesota, Arizona, Chile, California and Hawaii. The book provides a fine description of what it is like to visit and work at a great national observatory. There also are many instructive anecdotes, interspersed with amusing tales fit for telling on cloudy nights while the wind whistles round the dome. Of these, the best is Cohen's recollection of the invasion of an infrared observatory by a horde of moths, who so filled the optical path of the telescope that it was possible to sense their temperature if not, temporarily, that of the stars. I also was glad to find descriptions of the as yet inexplicable problems that intrigue the author, such as the circumstance that large and small stars seemingly are born in separate nurseries. Perhaps a young reader will some day find the answer.

This Quest lacks an account of teaching, which is a major responsibility for so many astronomers. Note also that the young readers inspired by Cohen will find fewer opportunities to enjoy the observatory life than did he; an "observing run" now often means a trip to a prosaic NASA or ESA



The Space Telescope — new opportunities for astronomers.

satellite control room. Further, in the 1990s, the data from the major mountain-based telescopes will surely flow to central city terminals or even directly to the "observer's" home institution, and jet-setting will fall off accordingly. High above, however, a few lucky astronomers will be operating their telescopes aboard the Space Shuttle.

Stephen P. Maran, a Senior Staff Scientist in the Laboratory for Astronomy and Solar Physics at the Goddard Space Flight Center, is currently helping to build a spectrograph for the Space Telescope.

# The chemists' Earth

Peter J. Smith

The Earth: Its Birth & Growth. By Minoru Ozima. Pp.117. ISBN hbk 0-521-23500-6; ISBN pbk 0-521-28005-2. (Cambridge University Press: 1981.) Hbk £10.50, \$22.50; pbk £3.95, \$8.95.

HERE is a cautionary tale. In 1947 the research vessel *Atlantis* dredged some metamorphosed basalts from the mid-Atlantic ridge at 30°N. When these rocks later came to be dated by the potassiumargon method they were found to be about 48 million years old, far from the roughly zero age they should have had on the basis of the seafloor spreading hypothesis just becoming popular. At the time this little inconsistency was put down to "error" (possibly the presence of excess argon-40, a well known age-invalidating phenomenon) and then conveniently forgotten.

Or rather it was forgotten by almost everyone but Minoru Ozima, who in the late 1960s had another go at dating the offending samples, this time using the new argon-argon technique with its isochron consistency test. In this way the correct age of the basalt was found to be 170 million years, thus producing even greater consternation involving accusations of specimen mishandling and cries of triumph from the "anti-spreaders". Observation and hypothesis were neatly reconciled in the end, however, by concluding that the basalt must have been left behind at the ridge from the time of the initial split of South America and Africa.

Only a geochemist would have the gall to embarrass the geophysical gods by dragging up this sort of thing; but the simple moral of the anecdote is well taken, namely, that isotope geochemistry has a vital role to play in elucidating the details of plate tectonic processes. What is more, unlike plate tectonic studies, which have hitherto concentrated largely on the past 200 million years, geochemical investigations are crucial in interpreting the whole course of the Earth's evolution. Earth scientists know this already, of course; but for the less specialist reader the more sober geochemical story has tended to fade against the background of the past two decades' blaze of geophysical publicity.

Ozima has set out to correct this unjust imbalance by providing for the general reader a short account of the way in which natural isotope studies have been used to trace the Earth's broad development. Beginning with the "birth of elements" in the nucleosynthesis that preceded the formation of the Solar System, he then looks in turn at the accretion of the Earth as a whole, the formation of the core, the separation of crust and mantle, subsequent changes in the crust, and the origin of the oceans and the atmosphere. The book ends with a discussion of the ways in which man-

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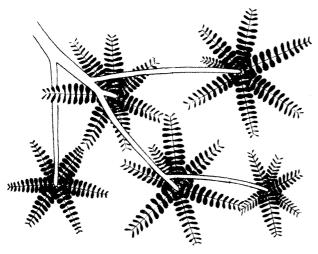
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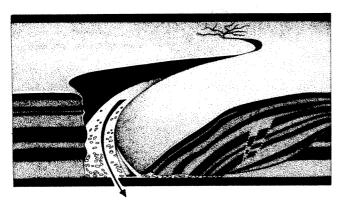
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made, high-level radioactive wastes might be disposed of in the Earth — a nice irony considering that the sites under consideration have been shaped almost entirely by the heat from radioactive isotopes, albeit different ones.

To a great extent, isotope geochemistry is a matter of dating events and processes that have self-evidently taken place or have been shown to do so using other types of evidence. This has tended to give the topic a "service industry" image, which is perhaps another reason why would-be popularizers have concluded that it lacks glamour.

Ozima's considerable achievement in this book has been to demonstrate to laypeople and students the vitality, excitement and importance of isotope geochemistry as a subject in its own right. In this cause he has been helped beyond measure by Judy Wakabayashi, who has produced a beautiful translation that puts the majority of native scientific English writers to shame.

Peter J. Smith is Reader in the Department of Earth Sciences at the Open University, Milton Keynes, and editor of Open Earth.

# Ultimate histories and final destinations

John Noble Wilford

Cosmic Dawn: The Origins of Matter and Life. By Eric Chaisson. Pp.302. ISBN 0-316-13590-9. (Atlantic Monthly Press/Little, Brown, Toronto: 1981.) \$14.95. Genesis: The Origins of Man and the Universe. By John Gribbin. Pp.360. ISBN UK 0-460-04505-9; ISBN US 0-440-2832-9. (Dent/Delacorte: 1981.) £7.95, \$13.95.

In content, purpose and intended audience (the educated layperson), these two books are much alike. Both cover the same ground, which is to say that they start with the big bang and bring us forward through the creation of the Solar System to the emergence of life and the ascent of man. But they differ considerably in style. Cosmic Dawn is an extended essay, instructive and literate, building toward a moving statement on the human condition and prospects. Genesis is a comprehensive exposition of current consensus and debate on our origins, tutorial in style but lively and often provocative.

Chaisson, an associate professor of astrophysics at Harvard, has produced a work of history as much as of science, with its narrative force and synthesis of grand themes. Astrophysicists, as he says, are the "ultimate historians". His essay is shaped around two "critically important transformations in the history of the universe". The first was the emergence of matter from radiation; the second, the emergence of technologically intelligent life.

The latter, he writes,

is the quintessential event in the development of matter, the threshold beyond which life forms can truly begin to fathom their role in the cosmos. Significantly, then, we have an obligation, a responsibility to survive.

Michael Ruse's *The Darwinian Revolution* (reviewed in *Nature* **284**, 670; 1980) has been published in paperback by Chicago University Press, price \$9.95 (US only).

To survive as a civilization Chaisson contends that we must circumvent the very real possibilities of overpopulation, selfdestruction, genetic degeneration and subjugation by computers. Long-term survival of the species, he states in an eloquent conclusion, may depend on our commitment to a two-fold programme of simultaneously colonizing the nearby planets, which he sees as a dispersal of the species so as to be less vulnerable to local catastrophe, and searching for galactic civilizations, which, if found, might reassure us that others have attained technological power and still avoided doomsday. The case for extraterrestrial exploration has rarely been better stated.

Gribbin, an astrophysicist and prolific science writer, also concludes with a forward-looking chapter, "Destinations", and suggests, among other things, the possibility of our descendants developing a "space culture". But, in general, his discussion of the future is unfocused and unsatisfying. This is a pity, for the rest of the book is so clear and rich in detail and insight. Much more than Chaisson, Gribbin spells out the discoveries and reasoning behind current thinking in cosmology and biological evolution. His discussions of plate tectonics, the origins of diversity and the origins of Earth are especially lucid. His style, like a gifted lecturer's, is by turns playful and authoritative. The index and organization by subheadings make it easy to refer back to subjects of particular interest.

Either book can be recommended to the student who seeks a broad understanding of the origins of mankind and the Universe (students of the humanities might prefer Cosmic Dawn), or to the general reader who wants a rewarding experience delving into the "ultimate" history.

John Noble Wilford is a science correspondent of The New York Times who specializes in space exploration. His most recent book is The Mapmakers (Knopf, 1981).

# Pressure to scale

W.D. Hackmann

Barometers. By Bert Bolle. Pp.256. ISBN 0-85242-710-7. (Argus, Watford, Herts: 1981.) £12.50. Early Scientific Instruments. By Nigel Hawkes. Pp.168. ISBN 0-89659-192-1. (Abbeville Press, New York: 1981.) \$29.95.

COLLECTORS of antiques are becoming increasingly interested in scientific instruments. Sundials, telescopes, microscopes and barometers are amongst the most popular. Barometers, especially, have commanded wide interest because of their attractiveness as pieces of furniture. Bert Bolle, a Dutch antique-dealer, has been a barometer enthusiast for many years. His book, which was first published in The Netherlands in 1978, occupies a niche between the purely technical histories, such as that written by W.E.K. Middleton in his The History of the Barometer (Johns Hopkins Press, 1968), and those describing antique barometers, of which the best English example is N. Goodison's English Barometers 1680-1860 (Cassell, 1977).

The book is divided into three parts. The first describes the evolution of the various barometer types, and each chapter deals with a specific pattern: stick, marine, miner's or pit, sign-post, banjo and aneroid. Ancillary instruments, such as thermometers, hygrometers and psychrometers, are also discussed briefly. Between 70 and 80 thermometer scales were in existence in the eighteenth century, and a Dutch example of 1754 with 18 scales is illustrated.

The earliest barometers were simple tubes containing mercury, based on Torricelli's well-known experiment of 1644, to which Descartes added a scale four years later. At first, the arrangement was used to demonstrate the "weight of air" but there was a slow realization that it could be used in forecasting weather. Research shifted to England. Both Robert Boyle and Robert Hooke improved the design, and gradually during the 1680s instrument-makers, clock-makers and opticians started to manufacture barometers for domestic use. Within a short period the main mercury barometer types were established. Trade concentrated in Britain, and to a lesser extent in The Netherlands, where a double or contra tube system (the contra-"bakbarometer") was invented by Huygens. Amongst the earliest makers were the famous clock-makers Thomas Tompion and Daniel Quare, then, during the eighteenth century, the manufacture of barometers became the speciality of expatriate Italian craftsmen; two of the best-known Victorian firms, Negretti & Zambra and Pastorelli & Rapkin, evolved in this way. In The Netherlands, too, Italian makers appeared in the 1750s.

The second part of the book is an illustrated catalogue of about 135 barometers. As expected, most examples are British, but France, Germany, The Netherlands, Denmark and Austria are also represented. Finally the author deals with the barometers and ancillary instruments encountered in the preceding parts from a technical point of view. Useful tables converting old into present-day units are given, and also recipes, for example for the camphor solution used in storm glasses. A novel arrangement of the book is that in each of the three parts the same chapter numbering is used when describing the same class of barometer. For instance, Chapter 3 in all three sections refers to the stick barometer. This facilitates crossreferencing, but also causes some duplication.

Michael Holford's beautiful photographs give the second book its obvious appeal. Nigel Hawkes, the science correspondent of *The Observer*, has been eclectic in his selection, ranging from a sixteenthcentury astrolabe to a late nineteenthcentury mahogany and brass telephone switchboard. The instruments can be seen in three London institutions: the Science Museum, the National Maritime Museum at Greenwich and the Royal Institution.

A remarkable feature of the book is the extraordinary size of the lettering, usually associated with books specially printed for the elderly suffering from myopia. No doubt this was necessary so that the somewhat bare text could fill the large page size required for the plates. The descriptions given are reasonably accurate, indeed in a few instances more correct than the museum labels exhibited with the original instruments; but this is above all a picture book, which gives only haphazard glimpses into a fascinating history.

W.D. Hackmann is Assistant Curator of the Museum of the History of Science, University of Oxford.

# History through the looking glasses

Silvio A. Bedini

Collecting Microscopes. By Gerard L'E. Turner. Pp.120. ISBN UK 0-289-70882-6; ISBN US 0-8317-5950-X. (Studio Vista, London/Mayflower, New York: 1981.) £2.95, \$14.95. The Camera Obscura: A Chronicle. By John H. Hammond. Pp.182. ISBN 0-85274-451-X. (Adam Hilger, Bristol/Heyden, Philadelphia: 1981.) £13.50, \$33.50.

As Gerard Turner states in his introduction to *Collecting Microscopes*, "A book for collectors of microscopes must be both a history book and a guide book". It must not only describe the instrument's development, but also identify the leading makers and the capacity of the instrument in its various forms to perform its scientific tasks. This is indeed a large order for a volume of modest size, but the author manages to meet his own requirements extremely well.

The microscope has had a long and complex history in evolving from the simple instrument of the early seventeenth century to the modern electron microscope. It was an endeavour in which many countries took part and to which its users — anatomists and other men of science — made as substantial contributions as did the makers of the instruments. It is not surprising that in time the microscope became the universally recognized symbol of science because it had such a wide range of application, from medicine to geology and from botany to metallurgy.

The book ranges from descriptions of the optical structure of the microscope, the anatomy and materials, to individual chapters on the several basic structural forms. Special attention is given to the English and Continental microscopes produced in the nineteenth century, the period which saw its greatest development. Noteworthy are sections on special hints to collectors, names and addresses of the most important museum collections throughout the world, a guide to prices based on auction and other recent sales, and a comprehensive bibliography. The work is illustrated by splendid pictures, many in colour, and by numerous diagrams.

The author is recognized as one of the world's foremost authorities on the history of the microscope, and is the author of many writings on the subject. His early training as a research physicist and his museum experience contribute greatly to this attractive, authoritative volume, which no collector of microscopes, historian of science or museum curator can afford to overlook.

Hammond's history of the camera obscura is also a welcome addition to the literature of optical instruments; again, it will serve not only the collector but the historian as well. In this informative volume, Hammond traces the history of the instrument from its use by fifth-century Chinese philosophers through Greece to Europe, where it was known by the thirteenth century.

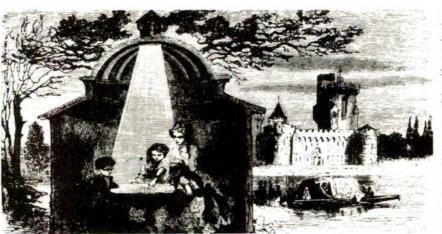
Simply stated, a camera obscura is a darkened room or an enclosed box into which light passes, generally through a lens, to form an image of external objects on the opposite surface. Gemma Frisius, Nicolas Copernicus, Tycho Brahe, Moestlin, Johannes Fabricius and Johann Kepler were early users of the instrument for observing eclipses, sun spots and planetary movements. The first portable model appears to have been designed by Robert Boyle and it was immediately imitated by others. Kepler devised a portable form in a tent which he probably used for his survey of upper Austria as well as for observing astronomical pheno-

The camera obscura progressed from serious scientific — and artistic — use to a device for public entertainment by magicians and other performers, and eventually was reduced to a parlour diversion. It was the subject of an extensive literature during the period of its maximum popularity, but more recently it has merited little more than passing mention in histories of science, art and photography.

In addition to a lucidly written, wellillustrated history, Hammond provides appendices and lists of references at the end of each chapter, as well as a comprehensive bibliography and full index.

Both of these authors have succeeded admirably in their aims; their books are contributions to the scientific literature as well as most useful and attractive guides for the collector.

Silvio A. Bedini is Keeper of Rare Books at the Smithsonian Institution, Washington DC, and specializes in the history of scientific instrumentation and horology.



The camera obscura as a diversion for children. The picture comes from F. Marion's Wonders of Optics (1868).

From The Camera Obscura, courtesy Science Museum, London.

# Entry to the history and philosophy of science and medicine

Donald Cardwell

Dictionary of the History of Science. Edited by W.F. Bynum, E.J. Browne and Roy Porter. Pp.496. ISBN UK 0-333-29316-9; ISBN US 0-691-08287-1. (Macmillan Reference Books, London/Princeton University Press: 1981.) £17.50, \$40.

THE lexicographer is, according to a high authority, a drudge. But this description hardly fits the three editors of the Dictionary of the History of Science. All are active scholars in the history of medicine and they have had the assistance of no fewer than ten subject editors. The title of their dictionary, however, requires amplification. What we are given is, in fact, an encyclopaedia of the history and philosophy of science, comprising short articles on general topics of between 50 and 2,000 words, interspersed between about three times as many brief references to specific points in the short articles which, in effect, constitute an index. The form of the work is, then, a cross between an encyclopaedia and a dictionary with the emphasis on the former. Few archaic or obsolete words are included; alembic. athanor, pelican and that legion of prescientific chemical terms are absent.

In short, this dictionary is more didactic than narrowly informative and it reflects a very definite view of the history of science. Technology is confined to one article while the philosophy of science and the history of medicine are well represented. This policy must have entailed problems for the editors barometer, cloud-chamber, galvanometer, thermometer all qualify for inclusion, while ammeter, voltmeter, bridge (electrical) do not. Presumably the latter were considered technological and therefore outside the scope of the Dictionary. Clearly, there is a need for a companion volume on the history of technology.

A perusal of the brief references is not without its lighter moments. Anyone who is not a demographer must be intrigued by the reference: "average man. See statistics (vital)". On the other hand, "improper egg. See generation" calls to mind an entry in that incomparable index to Wyndham Lewis and Charles Lee's anthology, The Stuffed Owl, "Eggs, mention of, wrapped in elegant obscurity, 62". The reference, "women. See chlorosis; hygiene; malefemale differences" will annoy militant feminists as well as invite comparison with another entry in the aforementioned index, "Woman, useful as a protection against lions, 118". As for "museums. See fossils", this suggests a particularly sour opinion of museum curators.

On a more serious note, however, I must take issue with the editors on a number of points. Some of these are minor and amount to little more than expressions of opinion. Surely, for example,

Newtonianism merits more than 13 lines? And should not mention of Gibbs and Heaviside be included in any account, however short, of the history of vectors? The British Association is, very properly, included but not the older Deutsches Naturforschers Versammlung (1822). Again, hospitals find a place but there is, unaccountably, no room for laboratories. And for their part, Mancunians must regret that no reference is made to daltonism.

Most serious, in my view, is the failure to mention units and dimensions. The development of the theory of dimensions and the establishment of rational units were, taken together, a most important feature of the growth of physics in the past 200 years. The British Association Committee on electrical units (1861) may have been a response to the needs of telegraph engineers and the results of its labour may have been vital for the establishment of the world's electrical supply industry, but they were hardly less significant in the realm of "pure" science, in the formulation of electromagnetic field theory. After all, Maxwell was a member of the Committee and his seminal paper was published in 1865.

On the other hand, when we consider the articles that are included we are amply compensated. Apart from a few brief passages about contemporary and fashionable theories of science, that may well prove ephemeral, the short essays that form the bulk of the Dictionary cover an enormous variety of subjects, ancient and modern. These range from Ptolemaic astronomy and Islamic science to matrix mechanics and causality in quantum physics, from Aristotle's cosmology to the paradox of confirmation, from Naturphilosophie to molecular biology and the genetic code. The Dictionary, in other words, provides material for a comprehensive course on the history and philosophy of science.

The omissions can easily be attended to in future editions and, all things considered, this is a valuable work. It is well produced and the individual articles, by nearly 100 scholars, are authoritatively, carefully and clearly written. Every library and every scientific establishment should have a copy.

Donald S.L. Cardwell is Professor in and Head of the Department of History of Science and Technology at UMIST, Manchester.

# Plants in print: more of Thornton's Flora

S.M. Walters

The Temple of Flora. Introduction by Ronald King. Pp.112. ISBN UK 0-297-77984-2; ISBN US 0-8212-1128-5. (Weidenfeld & Nicolson, London/New York Graphic Society, Boston: 1981.) £18.50, \$35.

THE spate of handsomely produced botanical and horticultural books continues unabated, in spite of the recession, and even professionals find it difficult to pick their way through them. A new edition of Robert Thornton's Temple of Flora, containing a complete set of reproductions of the famous colour engravings, is, however, a publishing event overshadowing most of the rest especially at such a remarkably modest price. In a detailed introductory section, Ronald King, a former Secretary of the Royal Botanic Gardens at Kew, tells the story of this extraordinarily grandiose venture by Thornton, who dissipated a private fortune on his scheme in the first years of the nineteenth century. King's introduction goes further back than the reader might expect, beginning with the birth of Linnaeus, and tracing the rise of Linnaean botany in the second half of the eighteenth century and its relation to botany and horticulture as relaxations and

hobbies enjoyed by Queen and humble citizen alike.

Thornton received an important part of his education in Cambridge, where he obtained his medical degree in 1793, and came under the influence of Thomas Martyn, Professor of Botany, a great supporter of the new Linnaean systematics. His career in medicine seems to have been both unorthodox and relatively unsuccessful. The evidence is that he had conceived something of his grand plan for a magnificent book on Linnaean botany as early as 1791, whilst still at Cambridge in fact, although it was six years before his circumstances enabled him to launch the project. The inglorious failure of the whole scheme in the "Royal Botanic Lottery" of May 1813 is described in detail; it is a quite extraordinary story, from which Thornton emerges as a figure to be pitied or despised according to taste. He was never to know that the third volume of his great work would achieve permanent and international fame - a "lasting heirloom for the British nation", as King says.

The generous layout of this book involves some repetition, particularly between the text accompanying each plate and the relevant page in the Introduction, and the reader should be warned that

important information about the plates may only be found in the introductory reference. As an example, the botanical identification of the plates, a technical and specialist matter, is not systematically included in the legends, although it can (in all cases?) be gleaned somewhere in the Introduction, Proof-reading seems to have been very good, though a small error occurs on p.16 in the title of Sir John Hill's Flora, which is given as Flora Botanica — a particularly odd mistake, since Sir John himself had mis-spelt his own title by calling the work Flora Britanica.

An adequate bibliography provides access to all relevant works including the most recent (1972) edition of the Temple of Flora, published by Collins and now out of print. Purchasers of this new edition may find it interesting and instructive to consult the Collins book in a library; in it Geoffrey Grigson gives a more concise account of Thornton's work which is also less tolerant of his vanity, greed for success, taste for bad poetry and generally (to us) his excessively "purple" prose style than is that given by King. Yet both agree, as the whole botanical world does, that the plates are superb. The publishers are to be congratulated on making them available again.

S.M. Walters is Director of the University Botanic Garden, Cambridge, and author of The Shaping of Cambridge Botany (Cambridge University Press, 1981).



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# The flowering of botanical illustration . . .

Sandra Raphael

The Art of the Botanist. (In the USA The Art of the Plant World.) By Martyn Rix. Pp.224. ISBN UK 0-7188-2482-2; ISBN US 0-87951-118-4. (Lutterworth, London/ Overlook Press, New York: 1981.) £25, \$60 until 31 December, £30, \$75 thereafter. Curtis's Flower Garden Displayed. Descriptions by Tyler Whittle and Christopher Cook. Pp.258. ISBN 0-19-217715-X. (Oxford University Press: 1981.) £19.50,

THE best botanical illustrations, most obviously the greatest eighteenth-century ones, transcend their strictly utilitarian purpose of recording the appearance of plants and become works of art in their own right. Dr Rix's book is called The Art of the Botanist, and its large format seems to give priority to the pictures; but the text that accompanies them is more like a frustrated account of botanical exploration than, as is intended, a history of botanical illustration. In an ideal world, this subject would be treated by an author equally at home with botany, bibliography and art history. Dr Rix is primarily a botanist, and the book reflects this lack of balance, for he is obviously more comfortable once he reaches the eighteenth century and the start of modern botany.

The first quarter of the book skims over the earliest manuscript and printed herbals and plant pictures, ending with a chapter on exploration in the sixteenth and seventeenth centuries and the books that hold the records of the new plants of the period. The next, and longest, part of the book deals mainly with the eighteenth century, perhaps the greatest age of botanical illustration, with a geographical arrangement leading to a tangled treatment of chronological developments. The final section starts as lithography begins to take over from engraving and covers nineteenth-century publications and the merest handful of twentieth-century work.

The pictures are, of course, the book's raison d'être - 250 of them in black and white and 64 in colour, all in only 220 large pages. They are beautiful, and the photographic quality is generally good; but the book's design does not give them the setting they deserve. The pages are packed, but the demands of colour-printing mean that pictures are often some distance away from the relevant text. No indication of the size of the originals accompanies the reproductions, so that those unacquainted with the books concerned are given no idea of their scale. Black frames surround nearly all of the pictures, a real distraction when several are printed on one large page, when the effect produced is that of a sheet of large stamps. The colour plates on pages 160 and 161 make a really unhappy pair, the strelitzia from Redouté's Les Liliacées at its best a clownish plant and hardly a beauty - facing the night-blooming cereus, complete with romantic background, "moonlight by Pether' from Thornton's Temple of Flora. Both of these would look infinitely better with more compatible neighbours.

An advertisement for The Art of the Botanist has described it as a definitive history of its subject. Even if one makes allowances for the context of such a statement, it is one to be treated with great caution. The book's bibliography is absurdly inadequate. Many important sources of relevant information appear to have been ignored, not least the Calmann study of G.D. Ehret, published in 1977 and the standard work on one of the greatest of flower painters. Dr Rix says a good deal about the Duchess of Portland's collection of Ehrets, although he is apparently unaware that two volumes of them have recently been identified in Oxford. He also mentions the Windsor copy of an Aztec herbal of 1552, "in which only one or two of the plants are actually recognisable", without troubling to tell us that the Windsor herbal is apparently a copy of the Badianus manuscript in the Vatican Library, published in facsimile in 1940 by E.W. Emmart, who also provided copious notes in which several of the plants are identified. The Plantin collection of plant illustrations, the source of so many of the pictures in sixteenth- and seventeenthcentury herbals, is given a few lines, but the recent rediscovery of it in Cracow, after its removal from Berlin during the last war, goes unnoticed. So one could go on. In addition there is the usual crop of minor misprints, among others, that hardy annual that turns the Pierpont Morgan Library into the Pierpoint Morgan.

Dr Rix's own travels have taken him to some of the regions explored by earlier botanists, and his book is most vivid when he can add his own first-hand comments to theirs. A member of Tournefort's expedition discovered a large vetch, apparently encountered by Dr Rix too, for he tells us that its "succulent young shoots" are "still much eaten by shepherd boys"

A picture book then, but not a definitive history of botanical illustration. For the nearest approach to such a thing we must still rely on Wilfrid Blunt's The Art of Botanical Illustration (1950) - much quoted by Dr Rix — and Claus Nissen's Die Botanische Buchillustration (1966)

Curtis's Flower Garden Displayed includes 120 plates chosen from about a thousand printed in Curtis's Botanical Magazine during its first 20 years (1787-1807) and 25 volumes. periodical, now not far short of its bicentenary, was founded to describe garden and greenhouse plants, each plate being accompanied by a short note giving a description, a potted history and notes on cultivation. The plants selected by Tyler Whittle and Christopher Cook have been arranged in order of their introduction to Europe, with the original text replaced by a new one, mixing botany, gardening and historical chit-chat with quotations from Turner, Gerard, Parkinson, Curtis himself and other botanical classics.

In Lib. primum Dioscoridis. 215



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"Rhus", possibly the stag's horn sumach (Rhus typhina). Woodcut from Commentarii in Sex Libros Pedacii Dioscoridis, 1565 edition, by Pierandrea Matthioli.

The arrangement echoes that of the Botanical Magazine itself, each plate facing its appropriate text. The plates are good copies of the originals, and the book is a very attractive production, making it even more of a pity that such a pleasant piece of printing is spoiled by extremely careless copy-editing and proof-reading. Misprints are numerous, perhaps the most comic being "naval wort" for navel-wort. Several personal names appear in variant forms; even Linnaeus's are given in the wrong order: Linnaeus was his family name, and he did not become von Linné until he was ennobled.

This selection of early Curtis plates will be welcomed by gardeners interested in the history of the plants they grow. It might even lead them to a full set of the *Botanical Magazine* itself, or perhaps to the dozen plates from recent issues sold as a calendar at Kew each year — a pleasant annual glimpse of some of the best of today's botanical illustration.

Sandra Raphael is Senior Editor (Natural History) in the Dictionary Department of Oxford University Press. She is currently working on a new edition of Wilfrid Blunt's The Art of Botanical Illustration.

# . . . and of the garden at Cambridge

Blanche Henrey

The Shaping of Cambridge Botany: A Short History of Whole-Plant Botany in Cambridge from the Time of Ray into the Present Century. By S.M. Walters. Pp.121. ISBN 0-521-23795-5. (Cambridge University Press: 1981.) £17.50, \$42.50.

This slim quarto volume was published to mark the 150th anniversary of an Act of Parliament, dated 30 March 1831, authorizing the acquisition of land from Trinity Hall for the development of a New Botanic Garden. By that time the eighteenth-century Walkerian Garden founded in 1762 had become too small, and, in the words of the Professor of Botany, J.S. Henslow, "utterly unsuited to the demands of modern science".

The book gives a brief and interesting history of the scientific study of botany in Cambridge; and the author, though not going very deeply into his subject, provides the botanist with a useful and handy source book. Moreover, at the present time when the study of garden history has such a wide appeal, a publication with special reference to the teaching, planning and research, carried on over the years in the garden at Cambridge, offers much of interest to the garden historian.

Dr Walters, at one time Curator of the Cambridge Herbarium and Lecturer in the Botany School, and now Director of the Garden, has written this book chiefly from material obtained from published works, from some unpublished manuscripts and from knowledge that he has acquired over many years of association with the University. It consists of a history of botanical and horticultural science in Cambridge from the seventeenth century, when John Ray established the tradition of herborizing, or plant hunting expeditions. until the establishment of specialized modern branches of botany, such as genetics and ecology, in the present century.

The eight chapters cover various stages in the development of the Garden and Cambridge botany. Details are given of the lives of the important figures in the history of the science and the part that some of these men played. Among them, from the time of Ray until 1825, are Richard Bradley, and John and Thomas Martyn, and from 1725 onwards, John Steven Henslow, Charles Cardale Babington, Sydney Vines, Richard Irwin Lynch, and Harry Marshall Ward and his successors.

A chronology at the end of the work, giving dates of historical events, lives of personalities, publications and so on, would have been a useful addition. In compensation, a feature of the book is the large number of illustrations — 84 in all — depicting title-pages, frontispieces, maps, portraits, plants and other subjects. There are also illustrations of specimens from

Ray's own herbarium, from Martyn's herbarium, and an illustration of an herbarium specimen Silena maritima made by James Donn. Furthermore, a number of figures of plants are reproduced from original drawings made specially for the work by Michael Hickey. Many of the figures occupy the wide margins of the book, enlivening the pages and creating a unity between text and illustrations. A coloured reproduction of Rosa "Cantabrigiensis" from a water-colour drawing by Graham Stuart Thomas, Gardens Consultant to the National Trust, makes a charming frontispiece.

Blanche Henrey is an Honorary Associate of the British Museum (Natural History), and author of British Botanical and Horticultural Literature (Oxford University Press, 1975).

# Britain in leaf

Alan Mitchell

A SINGLE volume on trees cannot, unlike one on birds, extend its coverage to the Mediterranean shores without losing much of its use in British gardens. The Hamlyn Guide to Trees of Britain and Europe by C. Humphries, J.R. Press and D.A. Sutton (Hamlyn; hbk £5.95, pbk £3.95) is a good example. Of the total of 20 species of pears and eucalypts covered, 15 are not grown in Britain. To encompass these and some obscure southern oaks and maples, the authors have had to omit important trees like Low's and Nikko firs, and, worse, ignore all the cultivars of Lawson and Sawara cypresses which crowd our parks and gardens. The book, then, will be of most use in the field in southern Europe. It has a good, workable synoptic key, and excellent descriptions and illustrations of details, though the whole-tree drawings are seldom in the least helpful and a few are quite wrong.

Alan Fairhurst and Eric Soothill's Blandford Guide to Trees of the British Countryside (Blandford, £9.95) contains beautiful photographs of the bark, foliage, flowers and fruit of the 55 trees, 9 shrubs and ivy which are given full treatment, and of a few of the 86 trees and 18 shrubs more briefly described or just mentioned. This last, however, is quite pointless when so many common trees are omitted altogether. The marginal outline drawings are reasonably distinctive (but "Dutch elm" is plainly English elm). The strength of this book, and exceptional feature, is the long lists of fungi and insects associated with each species, and of local names for the trees. The entries for timber uses and the general information are also good value.

A particularly handsome but unpretentious little book is The Guinness Book of Trees (Guinness Superlatives, £4.50). Here, Jeanette and Esmond Harris give a useful summary of the history of trees in Britain from pre-glacial times until the present, and raise a question-mark against the native status usually accorded to the beech and hornbeam. They select 18 conifer and 32 broadleaf trees for full treatment and full-page colour illustration, mostly of high quality and of fine specimens. The order in which the species are presented seems to have no rationale but there is much interesting information about each.

Unfortunately, the maps are a disaster area. That showing the areas of the world from which most exotic species are derived shades the wrong side of South America and even manages to omit California. Oregon and Washington as well as Korea; and the map of tree-collections puts Edinburgh north of Stirling and Oxford in Cambridge. The distribution maps, in bold red and white, even for exotics planted widely, are equally eccentric. Ireland is deprived of grey poplar (the largest specimens in the British Isles are there) and Norway maple, but is covered in English elm. Nonetheless, the line-drawings of foliage are neat and attractive and the book should attract its fair share of buyers.

The International Book of the Forest (Mitchell Beazley/Simon & Schuster; £14.95, \$35) is one of those books of large format and prolific illustration in which information from the text is repeated in boxes and captions, as if to cater for those who cannot face reading the whole tome. The scope in subject, time and region is enormous — from Pangaea through mediaeval forests to modern logging; birds, nocturnal creatures and forest man, to fire and the ecosystem, a single, double-page spread for each. Forest products also take a spread each but while elaborate maps and tables of the world timber trade may have their uses, the details of boat-building and of the manufacture of rubber tyres and plastics are out of place in a book on forests which can spare only a small box for the subject of tree-breeding. The main feature of the book is the surveys of forest types of the world, including the oceanic islands and mangrove swamps, and these include some magnificent photographs.

In complete contrast, as befits its different function, is *The Reader's Digest Field Guide to the Trees and Shrubs of Britain* (Reader's Digest Association, £6.50), a stoutly bound, attractive book in an unusual broad, shallow format. It is profusely illustrated with mostly good paintings and drawings, and one high-quality photograph per page, which last is, however, too small to be of much value. Arranged according to the shape of the leaf, the plants include a wide range of shrubs and yet there is still room for a good selection of trees and some cultivars. A

broad ecological slant is given by additional coverage of the wildlife in oak woods, beech woods and other forest types. There are five spreads depicting winter shoots and two on cones, and a small section on some of the places in which to see a variety of trees. Produced to the

usual standards of Reader's Digest books, this is a good specimen of the field-guide genre and a reasonable buy.

Alan Mitchell is a Dendrologist with the Forestry Commission and author of a number of books about trees.

# Arboreal splendours and uses

Richard Howard

The Oxford Encyclopedia of Trees of the World. Consultant editor Bayard Hora. Pp.288. ISBN 0-19-217712-5. (Oxford University Press: 1981.) £12.50, \$19.95.

"Ask a child to draw a tree and he will start with the trunk, then add a fan or brush of branches". A straight, woody, selfsupporting trunk, unbranched for a distance from the base, defines a tree at least 21 feet in height to most people. Yet the botanist or the horticulturist may be uneasy with such a definition, which in this book covers ferns (to 80 feet tall), cycads, grasses (bamboo), dragon trees (supposedly 6,000 years old), tree cacti (to 66 feet), as well as the broadleaved flowering plants and the cone-bearing gymnosperms with needle-like foliage. Trees in cultivation, the primary subjects of this volume, have a history as well as individual beauty and are useful to mankind for their bark, wood, leaves, flowers or fruit.

Thirty-nine authors excel in describing. distinguishing and illustrating the 350 species covered in this volume. The "main" species of large genera are grouped together, with notes on their key characteristics, permitting the reader to identify a plant and to understand the morphological variation within the genus. Useful keys for the identification of trees are provided for conifer families and the conifer genera by families, for the broadleaved families and aberrant genera, and for the broadleaved genera by families for those plants covered in the book. The introductory essays on trunk and wood structure, the forest ecosystem, the forests of the world, and forestry practices and products are instructive evaluations of the economic problems faced by a society which is still dependent on the tree.

The photographs, maps of distribution and drawings are well done, though there are a few exceptions. The photograph of *Cornus kousa* is a disappointing representation of that handsome flowering tree, and no one would understand the attraction of the American flowering dogwood or the Pacific dogwood from the short, leafy branches used as illustrations here.

The volume is not an "encyclopedia" of trees of the world, as titled, but is certainly a handsome compilation of "trees of many kinds, primarily those grown in Europe". The treatment of the gymnosperms (conifers) is the best ever published and alone is worth the price of the book. In contrast, the section "Trees of the Tropics" is atrocious, with errors of fact (e.g. mace, Bertholletia), identification (plate of jackfruit, text on Hura crepitans), and omissions of interesting or important trees (litchi, Blighia), and with a vocabulary inconsistent with the glossary or the other articles. Fortunately it is short and, if ignored, will not detract from what is otherwise an excellent publication.

Richard Howard, formerly Director of the Arnold Arboretum, is Professor of Dendrology at Harvard University.

# Anatomy of biology

Peter R. Scott

Biology in Profile: A Guide to the Many Branches of Biology. Edited by P.N. Campbell. Pp.128. ISBN hbk 0-08-026846-3; ISBN pbk 0-08-026845-5. (Pergamon: 1981.) Hbk £8, \$19; pbk £3.95, \$9.50.

WHAT should you tell an intelligent 17-year-old about the biological sciences? How can you convey something of the range of the subject to a bright school-child faced with the many different courses which further education offers? According to the International Council of Science Unions, the proliferation of the biological sciences has now made this task too difficult for any individual to tackle. They have therefore asked a number of distinguished biologists to explain something of their own areas of specialization; the result is this collection of 20 essays. The subjects covered may all be studied at university level, and range from zoology and botany to the less-familiar psychology, endocrinology and biophysics.

The authors were asked to address themselves to intelligent 17-year-olds who are already studying biology. There is no attempt at any consistency of style; some have needed to explain the background to their subject in detail, others have chosen

to relate some of the more spectacular discoveries of the past 20 years. Many give their own personal view of where the subject might lead in the future, and what it might offer to those who follow it.

A brief essay is not a suitable format for a careful weighing of complex arguments; rather it provides an opportunity for an optimistic and sweeping review of a field. Most of the authors have responded with a light approach — nutrition is sold as a growth area — and this makes Biology in Profile suitable reading matter for spare moments. Many of the contributions assume little biological knowledge, and would also be appropriate for those thinking of taking up biology for the first time.

The treatment of biology as 20 disparate subjects may cause concern to some teachers; but overall the book does not create an impression of fragmentation. None of the authors is able to describe his own area without referring to its overlap with topics described elsewhere - indeed, the idyllic picture is drawn of scientific progress being made by the combined efforts of biologists, chemists and physicists, all working in close cooperation. Some students may be surprised to see the emphasis placed by many of the writers on quantitative work; those who see biology as the only science which does not require a firm grasp of mathematics would be well advised to choose their area of specialization carefully.

Perhaps the most surprising omission from this collection is any discussion of medicine or veterinary science. Most of those who enjoy biology at school will consider these as a career, if only because they imagine that they know what they entail. A direct comparison with the other branches of biology might have been helpful, and have removed some misconceptions.

Biology in Profile will find a place in many school libraries. It will be bought for those bewildered by the range of biological subjects available at university, but I think it should also be read by teachers seeking answers to the question "What is the point of science?", and even by non-biologists. May we hope for chemistry and physics in profile?

Peter Scott teaches science at Charterhouse School, Godalming, Surrey.

# Lightfinger

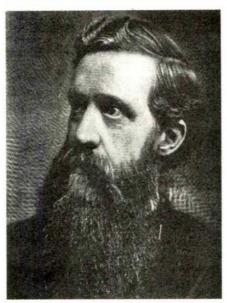
Following the success of the 1980 production, the Natural History Museum in London will this year stage "Lightfinger: A Space Fantasy for Christmas". The "fantasy" is designed for family audiences and takes the form of a science fiction comedy dealing with inter-galactic conservation and ecological problems.

The play will run from 18 December to 21 January (excluding 24-26 December and 1 and 11 January) with performances at 14.30 from Monday to Saturday, and at 15.00 on Sundays; special matinees for school parties start at 11.00.

# Elliott Coues, the combative naturalist

Clayton M. White

Elliott Coues: Naturalist and Frontier Historian. By Paul Russell Cutright and Michael J. Brodhead. Pp.509. ISBN 0-252-00802-2. (University of Illinois Press: 1981.) \$28.50, £19.95.



Elliott Coues - lover of birds yet sparrow hawk.

"A MAN with the mental calibre of Huxley and the charming personality of Mivart" — thus did Alfred Russel Wallace characterize the American ornithologist Elliott Coues; and Wallace's assessment was by no means unique. Also known for his contributions as a naturalist and frontier historian, Coues' achievements were recognized during his life (1842–1899) and he enjoyed ample publicity. The memory of his work has of course faded over the years, but this account of his life will do much to bring him back into the public eye.

Cutright and Brodhead have sifted a vast collection of hitherto unpublished material in the course of compiling their excellent biography. Capturing Coues in all his diversity, this book is for everyone; it is easy reading, yet informative and - not least — entertaining. Its 26 short chapters, refreshingly error free, draw heavily on the voluminous file of Coues' letters and his personal "Book of Dates". Each focuses on significant events in Coues' life: his time in Fort Randall (Dakota Territory), the expedition along the 49th parallel, the formation of the American Ornithologists' Union (Coues was one of the three founding fathers) and so on. Additionally, we are supplied with appendices and a bibliography of Coues' work.

Elliott Coues was an indefatigable worker and writer. His scientific career began at age 20, with a summer's excursion to the coast of Labrador. Funded by the Smithsonian Institution, he billed them the remarkable total of \$218.72 to cover his

entire summer's expenses. Three years later, as the result of work in the American south-west, he was firmly established as a naturalist; from that point his career was a steady upward climb to the apex of nineteenth-century American ornithology. He joined or was elected honorary member of 24 learned societies, among them the National Academy of Sciences, where, at 34, he was the youngest person to become a member.

His first published article appeared in 1861, even before his Labrador work; from then until his death he poured out more than 700 formal articles, edited works and other contributions ranging through science and medicine to theosophy. His literary skills matched his writing speed. His command of vocabulary, fertile imagination, ability to weave his exceptional observational acuity into the delightful fabric of his sentences and unexpected flashes of humour became trademarks of his work. To him, orioles were "gleaming through the sombre foliage like tiny meteors", whilst bushtits were "droll folk, quite innocent of dignity, superior to the trammels of decorum". Charles Lummis commented of Coues that

such a man never would have dried up to the proverbial scientific mummy. He humanized whatever he did without sacrifice of exactness. No one surpasses him in esoteric equipment; and as a 'readable' scientist, he was easily unrivalled.

While his prose would not be acceptable amid the stuffy curtness of our present scientific writing, I found it delightful:

We remember the 'rift within the lute'; in the canyon wren we have the lute within the rift — a curious little animated music-box, utterly insignificant in size and appearance, yet fit to make the welkin ring with glee.

And some passages cannot but elicit a chuckle. For weeks on end the scientific expedition along the 49th parallel had no fuel but buffalo dung for cooking and warmth. But Coues' pen was equal to the functionality of the dung:

as an agent in the progress of civilization...the buffalo chip rises to the plane of the steam engine and the electric telegraph, and acquires all the dignity which is supposed to enshroud questions of national importance or matters of political economy.

After obtaining both MD and PhD degrees, Coues was employed as an army surgeon. As such, he was often sent to remote western outposts; most of his time was spent in the pursuit of birds, however. Two of his works are directly attributable to this period: Birds of the Northwest and Birds of the Colorado Valley. Perhaps his major publication is his Key to North American Birds, an inspiration to such twentieth-century ornithologists as Ernest Thompson Seton and Frank M. Chapman. Of it, Seton wrote:

Faults it had in abundance, I now know; but it

was the first successful effort to take exact bird knowledge from the museum, and give it to the multitude, to place it within reach of all the world of those who love to hold our birds, not as skins, but as loving friends.

While birds were his main interest, he also collected other creatures. One of his prize finds was a rare western rattlesnake which he even stopped to collect while "under the untoward circumstances of a hasty retreat from hostile Indians". Although he often fought Indians, he eventually grew to champion them. While he saw what he considered defects or evils in their character, he concluded that

the Indian is neither a foreign power to be treated with, nor a wild beast to be hunted down, but a fellow-man to be reclaimed. Let us begin by calling him, that in the end we may make him, a brother.

Coues dabbled in theosophy and spiritualism. He became an exacting historian producing works on Lewis and Clark and other early western explorers, trappers and traders. But it is for his ornithological work that he will be remembered. His help to and influence upon Louis Agassiz Fuertes, undoubtedly America's greatest bird artist, is incalculable. And during his life Coues described 36 forms of bird and 18 forms of mammal new to science, many of them new genera and most of which are still recognized today.

Considering his intense and life-long love of birds, it is ironic that his most intense and persistent effort was an unrelenting war against one: the European house sparrow, recently introduced into North America. This particular bird he deeply loathed, and from the 1860s onward attacked it and its defenders with equal venom. It is doubly ironic that the main defender and thus combatant was Coues' friend, T.M. Brewer. Brewer was not spared abuse even after his death - "We . . . know that Brewer was a cantankerous old ass at the time he had the good taste to fall asleep in Jesus". Nor was Coues' work ineffective; eventually he was even accused of "treason" due to having "incited a riot" against the sparrow. But he yielded to neither the bird nor its friends; in 1878 he publically divided the bird's defenders into five categories, four of them composed of idiots and the fifth of the weak-minded.

In the long term, of course, his efforts were in vain; the sparrow has spread in North America from coast to coast and from central Canada to southern Mexico. And the final irony? Coues certainly would not have been pleased when, in 1975, the American Ornithologists' Union presented their most prestigious recognition, the Elliott Coues Award, to a team of researchers for their notable study on the evolution of the house sparrow in North

What's that (North American) animal?

Richard G. Van Gelder

The Audubon Society Field Guide to North American Mammals. By John O. Whitaker, Jr. Pp.745. ISBN 0-394-50762-2. (Knopf: 1981.) \$11.95. Harper & Row's Complete Field Guide to North American Wildlife. Eastern Edition assembled by Henry Hill Collins, Jr. Pp.714. ISBN 0-690-01969-6. Western Edition assembled by Jay Ellis Ransom. Pp.809. ISBN 0-690-01971-8. (Harper & Row: 1981.) \$12.95, £9.50 per volume.

THE purpose of a field guide is to enable one to identify an object without having that object in hand. This usually means that the criteria for identification are different from those on which the strict classification of the species is based. Nonetheless, some eminently successful field guides have been produced - birds, in particular, lend themselves to adequate field identification, to some extent because birds characterize themselves by sight and sound, two better-developed human senses. When it comes to animals with a greater dependence on odour or ultrasonics, such as shrews, rodents or bats, field identification becomes much more difficult. Despite the relative success of the "Peterson system" of field identification - drawings or paintings with "key" characteristics indicated, arranged for ready comparison — innovative attempts continue to be produced.

John O. Whitaker's Audubon Society Field Guide to North American Mammals is small enough to fit into a large pocket and is the first major attempt to utilize colour photographs for mammal identification. The 313 photographs (representing the 368 species, exclusive of whales and dolphins) are grouped rather sensibly by similar-sized and similarappearing species, even if unrelated; for instance muskrats, beavers and marmots appear together. While this may disconcert the taxonomist, it will probably aid the layman. The colour plates are followed by 403 pages of text dealing with each species, arranged in standard taxonomic order. These accounts include a description with italicized identifying characters and measurements, sign, breeding habits, habitat, range (with a small map) and excellent though concise life-history information. Marginalia often include drawings of animals not otherwise illustrated, and tracks. Appendices include a glossary and a complex "Range Chart" to



aid in the identification of some of the smaller mammals. The book is indexed and cross-referenced, but there is no bibliography.

Harper & Row's Complete Field Guide to North American Wildlife attempts to provide notes on and a source for the identification of birds, mammals, reptiles, amphibians, fish, and molluscs and other marine invertebrates of America north of Mexico. The two volumes cover the eastern and western USA, divided at the 100th meridian. This involves considerable duplication of species accounts as well as of illustrations, but not necessarily of



information — westerners are largely deprived of notes on the reproduction of mammals, for example. Within each species account there is a description, a comparison with similar species, and sadly variable and inconsistent information on habitat, habits, voice, food and range. Half of the total of 239 plates are in colour, and all are cross-referenced to the text (not always correctly or in the same volume), and "edge-marked". References are given in a section introducing each major taxonomic category.

Comparing the mammal sections, neither of these guides is superior for field identification to Burt and Grossenheider's volume, A Field Guide to the Mammals, in the "Peterson series", which has finer illustrations and maps. With Whitaker's book one is more likely to come up with a valid identification than with the Harper and Row volumes, but for most of the smaller species one has to resort to text descriptions and geographical information in both of these sources. Whitaker's small maps (without state or province boundaries) are useful, but the reader must be a good geographer to grasp the range of, for example, the Gray Myotis ("in se. U.S., from Ky., w. to Mo. and Kans., s. through Ala.") in a Harper and Row volume; the only map, on the inside of the front cover, does not identify the states.

For life-history information, Whitaker's book is the best single, concise source currently available, and for this reason is highly recommended. The larger Harper and Row volumes are a bit much for a pocket, and seem to have been planned by a committee to attract everyone, but satisfy no one.

Richard G. Van Gelder is Curator of Mammals at the American Museum of Natural History, New York.

Clayton M. White is a Professor of Zoology at Brigham Young University, Provo, Utah.

# Cyanide in Biology

edited by Birgit Vennesland, Eric E. Conn, Christopher J. Knowles, John Westley and Frode Wissing

December 1981/January 1982, xiv + 548pp., £22.00 (UK only)/\$45.50, 0.12.716980.6

This book, covering as it does all aspects of the metabolism of cyanide, should serve not only as an introduction to the uninitiated biologist, but also as a handbook for those already engaged in research in this area. This aim is undoubtedly helped by the extensive bibliography which integrates the widely scattered literature from the publications of different disciplines. Graduates and researchers in biochemistry, microbiology, plant physiology and toxicology alike will find this book valuable reading.

International Lecture Series in Computer Science

# The Correctness Problem in Computer Science

edited by Robert S. Boyer and J. Strother Moore

November/December 1981, xiv + 280pp., £14.20 (UK only)/\$29.50, 0.12.122920.3

This collection of papers describes continuing research by several internationally recognized authorities on the topic of computer program correctness. Program correctness is here viewed as a mathematical concern. To produce correct code the programmer should have at his disposal the same kinds of formal and informal tools used by mathematicians. These papers describe research by the authors aimed at producing such tools, including design methodologies, formal specification languages, mechanical program verifiers, and mechanical theorem-provers

# Cytochrome Oxidase

A Synthesis

Mårten Wikström, Klaas Krab and Matti Saraste

December 1981/January 1982, c.200pp., £14.60 (UK only)/\$30.00, 0.12.752020.

This volume provides an up-to-date synthesis of research material on cytochrone oxidase. Various models and hypotheses are used as frames for the experimental data, and themes covered include protein structure and topography of the enzyme in its isolated and membrane-bound states; physical properties; configuration and topography of the haem and copper centres, their interactions and oxidoreduction properties under equilibrium conditions; kinetics and catalytic mechanism of electron transfer and oxygen reduction; mechanism of energy transduction by proton translocation

# Honey Bee Pathology Locomotion

Leslie Bailey

December 1981/January 1982, x + 132pp., £9.40 (UK only)/\$19.50, 0.12.073480.X

The book gives a comprehensive account of all the important and common pathogens and diseases of the honey bee, with emphasis on modern knowledge of their natural history, especially of their ecology within the insect society, and with attention to their place in the pathology of Insects in general. Diagnostic methods, including previously unpublished techniques for cultivating and purifying microbial pathogens and viruses are described; and treatments for diseases are reviewed and discussed.

# **Bioenergetics**

An Introduction to Chemiosmotic Theory **David Nicholls** 

January/February 1982, c.180pp., Casebound: £13.40 (UK only)/\$27.50, 0.12.518120.5 Paperback: £5.50 (UK only)/\$11.50 0.12.518122.1

In the 1960s. Peter Mitchell put forward a theory which revolutionized our understanding of bioenergetics. Called the Chemiosmotic Theory, its essence is fundamentally simple. Nevertheless, general biochemistry texbooks deal with it only superficially and the research literature is unapproachable for the uninitiated. This book has been written with the aim of introducing the subject from first principles, and then discussing particular aspects in sufficient detail to enable the reader to tackle the primary sources.

# **Biomass as Fuel**

L.P. White and L.G. Plaskett

November/December 1981, x+212pp., £14.20 (UK only)/\$29.50, 0.12.746980.X

Despite wide research and considerable advances, there have been few publications offering a comprehensive review of the subject in any depth. The authors of this book have identified this need and set out here the feasibility, time-scales and overall potential of the many possibilities. They explore the resources presently and potentially available and the techniques necessary to exploit them. These include animal and crop farming on land and short rotation forestry; the harvesting of natural vegetation; mariculture and the mass cultivation of micro-algae.

# **Energy Metabolism** of the Cell

A Theoretical Treatise J.G. Reich and E.E. Sel'kov

November/December 1981, c.302pp £36.00 (UK only)/\$74.00, 0.12.585920.1

This monograph is primarily a theoretical treatment of cellular energy metabolism, which requires a good background knowledge of biochemistry on the part of the reader, but a modest knowlege of mathematics. By searching for compact kinetic blocks of metabolites and reactions, the authors aim to simplify the complex schemes of biochemical pathways and present an integrated picture so that the performance of the system as a whole can be studied

Symposia of the Zoological Society of London

# Vertebrate

edited by M.H. Day

September 1981, xviii + 472pp. £37.20 (UK only)/\$89.50, 0.12.613348.4

This volume is based on a symposium held in London on 27th and 28th March 1980, under the joint sponsorship of the Zoological Society of London and the Anatomical Society of Great Britain and Ireland. The way in which vertebrates move constitutes a sector of animal biology that is of great evolutionary significance, as the ability of move in an effective way may be absolutely vital to survival and evolutionary

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# No sense and nonsense and noses

Bryan D. Turner

The Doomsday Book of Animals: A Natural History of Vanished Species. By David Day. Pp. 288. ISBN UK 0-85223-183-0; ISBN US 0-670-27987-0. (Ebury Press/Viking: 1981.) £14.95, \$40. After Man: A Zoology of the Future. By Dougal Dixon. Pp.124 ISBN UK 0-246-11577-7; ISBN US 0-312-01163-6. (Granada/St Martin's Press: 1981.) £8.95, \$14.95. The Snouters: Form and Life of the Rhinogrades. By Harald Stümpke. Pp.118. ISBN pbk 0-226-77895-9. (Chicago University Press: 1981.) \$4.95, £3.50.

CATS, rats, mongooses and above all mankind have caused over 300 extinctions of vertebrate animals during the past 300 years. This rate is far greater than estimates of that of the dinosaurs' extinction at the end of the Age of the Reptiles in the late Cretaceous period. David Day's The Doomsday Book of Animals describes something of the biology, in many cases all the known biology, of these 300 vanished species and subspecies, and chronicles their final days. The style is factual and unemotional, but the book generates a great feeling of sadness as page after page records loss after loss. Those perceptive of the value of retaining the world's biological diversity will feel something of the corporate guilt that rests on Homo sapiens as the destroyer of that diversity.

By far the greatest losses have been suffered by island communities, with only 75 animal species or subspecies disappearing from the continental land masses including Australia. Of these, 16 are fish which died out as their small "aquatic islands" in North America were tampered with.

Whilst the introduction of cats, rats and mongooses, whether intended or accidental, has logically resulted in the extinction of many rare endemic island species and subspecies, particularly in the West Indies and the Mascarenes, in other instances it is almost inconceivable that a species could disappear because of the sheer size of its population. The extinction of the passenger pigeon is perhaps the most astounding case since it was probably the most abundant bird species in North America. Flock sizes of between one and two million birds were reported not 50 years before the very last passenger pigeon died in Cincinatti Zoo in 1914. They were hunted relentlessly for sport, food and quack medicine, and as the flocks became more scattered so the telegraph system was used to direct the hunters' efforts. Man the hunter does not operate as do other predatory species which switch to alternatives when favoured prey are uncommon thus allowing the preferred prey to recoup its losses. Rather, he develops tunnel vision and having formed a search image is unable to switch to another prey until forced to

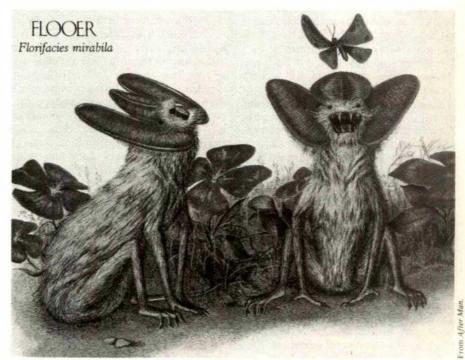
because that prey no longer exists. It is a characteristic of the examples in the *Doomsday Book of Animals* that as a species becomes rare through man's hunting pressure, so man's efforts against that species have tended to increase. Although there may be an awakening enlightenment that the world's diversity of species needs to be conserved, David Day's beautifully illustrated and poignant book ends pessimistically by reminding its readers that there are currently over 400 critically endangered species, many of which will become extinct in the near future.

When human beings themselves become extinct and take with them many of the groups of animals we know today, new life forms will evolve to fill the resulting ecological lacunae. This is the basis of Dougal Dixon's look into the future, 50 million years hence. Although After Man is fun, and displays some imagination, the biological framework within which it purports to operate is clearly little understood. In many cases the futuristic species just could not follow the lifestyle attributed to them. What happens to the leaping devil (Daemonops rotundus), a sort of carnivorous jerboa, after it has sunk its needle teeth into a large lizard? It has neither the apparatus nor the capacity to do much else unless it swallows the lizard whole and becomes the rolling devil. How does the gigantelope (Megalodorcas giganteus) actually eat the bulbs and roots it grubs up with its splendid horns, which extend in front and below the animal's muzzle and prevent its getting near the ground? The book is shot through with biological anomalies and lacks credibility despite the introduction by Desmond Morris, who should have known better than to attribute the book with "real scientific value".

Whereas After Man tends to be conservative in its conception of alternative life forms, Harald Stümpke's (alias Gerolf Steiner) zoological fantasy is highly inventive. Absent from the bookshops for the past ten years, the reprinting of The Snouters has been long overdue. It is an account of the adaptive radiation of a small group of insectivorous mammals which are characterized by the extensive development of the nose or nasarium. Among the 189 species in the order Rhinogradentia the nasarium is variously used temporary support while the legs serve for food processing; as an adhesive organ in the sedentary species; as a jumping organ in the hopsorrhines; and in the polyrrhines it becomes sub-divided into foot or tentacle structures. Throughout this bizarre but fascinating account the form and function are minutely examined in true Germanic taxonomic style.

Such realism will endear *The Snouters* to those with a zoological background but students should beware the rhinogrades, they are prime spoof material for inclusion in vertebrate courses. However it may be amusing to see whether straight faces can be maintained when the feeding habits of *Nasobema lyricum* are explained. This beast feeds on fruits which it reaches by, to put it politely, passing wind into its inflatable tail thus momentarily producing a long rigid appendage with a grasping tip.

Bryan D. Turner is a Lecturer in Zoology at King's College, University of London.



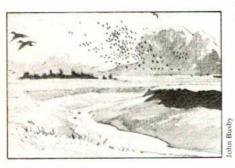
The insectivorous flooer (Florifacies mirabila). The animal has glands around its mouth which produce a sweet-smelling secretion and attract the flooer's prey.

# Some help for the dudes and praise to the experts

John Andrews

BIRDWATCHING beginners are badly served by publishers, their needs being met neither by the abundant field guides, which are mostly too wide-ranging and complex for the newcomer, nor by the glossy coeditions "packaged" to sell in several countries rather than to inform in one. One bright side to the recession has been that the latter have vanished from the scene, though it may be mere coincidence that two books have appeared which every beginner should own.

John Gooders' The Bird Seeker's Guide (André Deutsch; hbk £6.95, pbk £3.50) gives invaluable instruction on where to find birds in Britain. The opening chapters contain general guidance on the ways in which bird populations change with the seasons and on the characteristic group of species or



"bird community" to be found in each major habitat type. The main text comprises species accounts, each containing brief identification notes which concentrate on key points for separating similar species, and a description of the preferred habitats and distributions. Where necessary for uncommon species, likely locations are given but commendable care has been taken to avoid reference to precise sites in the case of vulnerably rare birds.

Anyone venturing into the field and encountering other birders will rapidly come up against the substantial jargon of ornithology, which ranges from arcane scientific terms to offensive slang. For instance, there are about 40 different areas of a bird's plumage each with its own name and one must know them all to make clear written descriptions of birds seen in the field: if you don't, you are clearly a "casual birdwatcher without serious commitment" or, more succinctly, a dude. No longer need you flounder in ornithology's information gap. The Birdwatcher's Dictionary by Peter Weaver (T. & A.D. Poyser, £5) defines over 1,100 terms and abbreviations, to enable the newly-fledged birder to match insult or erudition with equal ease. Crisply illustrated by Mike Hodgson, the text is thoroughly instructive and makes good browsing as well as useful reference.

By contrast with the needs of the beginner, scientific ornithology in Britain is usually well served by carefully researched and presented books. This year has seen only a single addition to the list but one eagerly awaited by the great number of amateur ornithologists who contributed to the Birds of Estuaries Enquiry, which ran from 1969 to 1975. Every winter for those six years the total wader populations of all the estuaries of Britain and Ireland were counted in perhaps the most ambitious and demanding ornithological survey ever undertaken. Substantial organization was needed to find and coordinate the teams working each area. Few of the thousand people who took part began as skilled wader counters, so techniques had to be evolved, imparted and checked for accuracy. Some personal commitment was also required: it is no light task to rise in the January small-hours, drive to a distant estuary for a dawn high tide and strive to count whirling flocks of birds, sometimes many thousands strong, with rainspattered lenses or frost-numbed fingers. Now the intangible and sometimes illusive rewards of the experience take a more practical form in A.J. Prater's Estuary Birds of Britain and Ireland (T. & A.D. Poyser, £14), which draws together the results of this mammoth study and much more information besides.

The opening chapters examine the nature of estuaries and the ways in which birds utilize them, including consideration of the factors which influence feeding success. Turning to migration and distribution in Western Europe, the author, who was the national survey organizer, discusses in particular the complexity of wader movements between estuaries which ringing work is now unravelling. Two chapters are devoted to threats to estuaries barrages and reservoirs, airports, agriculture, energy generation, pollution and leisure all figure. This is a slightly disappointing section of an otherwise excellent work, with too much emphasis on the nature and scale of impact and too little on the causes and justification - or lack of it - for the threats themselves: some startlingly costly and grandiose ways of wasting public money continue to be dreamed up for our estuaries. Returning to his main theme, Tony Prater describes the organization of the Enquiry, discusses count methodology and considers the vexed question of assessment criteria.

About half of the text comprises detailed summaries of present knowledge, both estuary by estuary and species by species. Copious maps and tables make much of the textual information available at a glance: there is an excellent bibliography, some photographs of marginal usefulness and many charming drawings by John Busby. Overall, a valuable book of a remarkable survey. Other nations please copy.

In turn, Britain, overrun by mink and, to some opinions, by Canada geese, might do



well to copy Western Australia in evolving a firm policy to control importation and translocation of potentially harmful wildlife. As a step in this direction, the States Agriculture Protection Board initiated a study which became, in the hands of John Long, the author of Introduced Birds of the World (David & Charles, £15), a comprehensive review of every known avian introduction worldwide. He found records involving 425 species and for each describes its natural and introduced range, the history and outcome of its introduction, and discusses its potential for damage. And damage there has been - extinctions of native species through competition and hybridization, substantial losses of agricultural and fruit crops and, possibly, the transfer of diseases and parasites. Western Australia is to be congratulated on the book: would our own agriculture departments were as wise.

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, Bedfordshire.

# The dissent of woman

Andrew Hill

On Becoming Human. By Nancy M. Tanner. Pp.373. ISBN hbk 0-521-23554-5; ISBN pbk 0-521-28028-1. (Cambridge University Press: 1981.) Hbk £20, \$29.95; pbk £6.95, \$10.95.

CHIMPANZEES are more like human beings than they appear. Linnaeus — the type specimen of taxonomists — originally classified the beasts in the human genus as *Homo troglodytes*, and scientists have continued to stress the similarities. *On Becoming Human* follows this tradition by proposing the chimpanzee as a model for the common ancestor of apes and man.

The book possesses a number of attractive features. Some of the latest evidence of affinity comes from work on proteins, and here we have a useful summary. There is also much on chimpanzee behaviour, emphasizing the

role of females in their societies, and in evolution as well. Importance is attached to social factors in evolutionary change, to cooperation rather than competition, and to the probable significance of plants both as food and as material for tool making. This information, along with details of fossil finds, is put together to provide a story of human evolution that more or less fits with what is known. But after all, so little is known — although this has never been a drawback for theorists in evolutionary anthropology.

In addition, the book has unfortunate aspects. Palaeontological objections to assessments of phyletic divergence times based upon protein work are not thoroughly explored. The description of chimpanzee behaviour is not adequately set in the context of broader primate ethology, and is needlessly anecdotal rather than being presented in a rigorous scientific manner. Given the aim to show the role of female sociability as a force in human evolution, the chimpanzee female is not a particularly good analogue, since its social network is more restricted than those of many other higher primates. Further, there are things to object to in the palaeoanthropological sections as well, such as an idiosyncratic view of stratigraphy.

Perhaps worst, the text itself has been seriously affected by the contemporary problem of inflation. The material could have been better organized, and the style is very repetitious (with frequent parenthetical interruptions) and often says the same thing twice (or more). The maps are so reduced that it is impossible to see whether they would convey information even if larger, and the illustrations are inappropriately cute and sketchy for work of whatever sort this is — indeed it is difficult to guess for whom the book is intended.

Models have a limited value; plausibility is about the only thing they satisfactorily demonstrate. They raise questions and illustrate possibilities, for it is likely that transitional hominids were at least as complex as chimpanzees. But they can produce a confining and chauvinistic outlook. Tanner appears to reject anything that smacks of reductionism, but surely more reliance can be placed upon conclusions derived from a larger taxonomic base, from a method that aims to detect general principles. What are the consistent social correlates of a long life, for example, or a long period of infant dependence, in a wider range of mammals?

Anthropological beliefs are the product of their time, and while attempting to expose this and the role of nineteenth-century British male prejudice, I feel that Tanner falls into much the same trap by infusing her treatment with the equally parochial attitudes of some contemporary American women.

Andrew Hill is a Research Fellow at Harvard University.

# Animal behaviour from A to W

Thomas E. McGill

The Oxford Companion to Animal Behaviour. Edited by David McFarland. Pp.657. ISBN 0-19-866120-7. (Oxford University Press: 1981.) £17.50. To be published in the US early next year.

THE 1973 Nobel Prize for Physiology and Medicine was awarded to the animal behaviourists Karl von Frisch, Konrad Lorenz and Nickolaas Tinbergen. That event was, of course, the major landmark in public recognition of the discipline. But it is still gratifying that Oxford University Press has selected animal behaviour as the subject of the first of their scientific "Companion" series.

The book "has been designed as a nonspecialist introduction to the study of animal behaviour". It contains an alphabetical series of 227 articles by McFarland and 71 other contributors. The essays, from Abnormal Behaviour to Wildlife Management (no Zoos?), range in length from a few lines - on, for example, immobility and gregariousness - to 18 pages on the history of animal behaviour. Many of the articles include excellent illustrations. The volume ends with a bibliography of 146 books and with indices of both the common English names and the scientific names of animals. Each index indicates the articles where a particular species is mentioned.

Six articles are devoted to people — the three Nobel laureates plus Charles Darwin, Ivan Pavlov and B. F. Skinner. The essays contain a brief biography and a discussion of the contributions of these luminaries.

While the book is designed for the layman, many will find it useful in teaching. For example, a two-paragraph article on vacuum activities provides some interesting examples and discusses how these behaviours might occur through stimulus generalization. A cross-reference leads to an article on generalization, containing other cross-references, and also a reference to the bibliography.

Aside from the inevitable unevenness of an edited work, the book has a major deficiency. It contains no listing of the titles of the articles, neither in a table of contents nor in an index. Thus one is left to guess the title of a particular subject. For example, while a scientific society and an international journal are called "Behaviour Genetics", there is no listing under that topic. Instead, the material is found under Genetics of Behaviour. There are no articles entitled Biological Clocks, Biorhythms or Circadian Rhythms; the relevant material is discussed in the contributions on clocks and rhythms. There is no entry for sociobiology (surely this topic deserves at least a crossreference).

Guessing and page-turning proved mildly annoying, so I made my own list of

the articles. I would advise others to do the same since this list proved a great aid in finding material and in browsing amongst unfamiliar topics. Certainly, there is treasure in this valuable book, but much of it is buried.

Thomas E. McGill is Chairman and Hales Professor of Psychology at Williams College, Williamstown, Massachusetts. He is editor of Readings in Animal Behavior, now in its third edition.

# The IQ agony aunt

Steve Blinkhorn

Straight Talk About Mental Tests. By Arthur R. Jensen. Pp.269. ISBN US 0-02-916-440-0; ISBN UK 0-416-32300-6. (Free Press, New York/Methuen, London: 1981.) \$12.95, £8.95.

WITH a publisher's blurb that would do credit to a headache remedy advertisement, and a shoulders-squared, plain brown wrapper of a title, Jensen's latest offering is a curious mixture. Part textbook, part highbrow agony column, it proclaims itself to be a plain man's guide and largely eschews mathematical formulae and reference to source material. But despite the obvious good intentions the book turns out to be no more than a competent rehash, lacking the brash vigour of Eysenck's brand of popular psychology and failing to compensate with novelty of argument or perspective.

What, after all, is there new to say about mental tests? Not enough, apparently, to merit a whole book, since the predominant theme of this one is the primacy and genetic determination of general intelligence, rather than the mental tests of the title. This is not to say that there is any evidence of lack of competence. As in Bias in Mental Testing (Free Press/Methuen, 1980), clearly the elder brother of this volume, Jensen shows considerable mastery of the issues. And when it comes to the evaluation of the role of IO measurement for practical purposes, there is no evidence of support for the kind of gee-whizzery and technical witch-doctory that mental tests can attract. It needs to be said by such as Jensen that tests are overused and often misused, but not thereby rendered useless, and he makes the point clearly and sensibly.

It is all the more difficult, then, to understand why he finds it necessary to pick continually at the scab of black-white IQ differences, why two of the six titles in the select bibliography concern race, indeed why he could not confine himself to the matter of mental tests, the jargon and technology of which are as often misunder-

stood amongst psychologists as in the world at large.

In the final chapter, switching from solid, textbook prose to a question-and-answer format suggesting the world's most degree-laden agony aunt, Jensen treats the reader to a glimpse into his postbag.

- "My father is a world-recognized genius. Yet I am sure he would flunk any IQ test. What do you think of that?"
- •"What can I do for my child to raise his IO?"
- o"Is our national IQ declining?"

No doubt these are genuine extracts from letters the originals of which are available for inspection at the author's premises. The format of this chapter certainly conveys the impression of an attempt to provide a plain man's guide, but at the same time shows up the failure of the enterprise as a whole. For what Jensen has done is not to strip off inessential technicalities to reach a wider audience, but to present himself as the man in the white coat and invite confidence in his knowledge and expertise. He lacks neither, but the suspicion always lurks that he is too much of an interested party to be wholly dispassionate.

Steve Blinkhorn, a Senior Lecturer in Psychology at Hatfield Polytechnic, is currently working at the Neuropsychology Laboratory, Stanford University.

# The naturalist's art

T.R. Halliday

ART and natural history have always been closely associated. But, over the past hundred years, illustrations have fulfilled a variety of functions in the natural history literature; the purpose of some is simply to aid identification, of others to be purely decorative and of yet more to encapsulate some aspect of the natural way of life of the creature portrayed. A number of recently published books not only enable us to trace the development of art in natural history from the nineteenth century to the present day, but to see how different artists have been concerned with one of these aims more than another.

A new, selected reprint of the Reverend F. O. Morris's British Birds, first published in parts between 1850 and 1857, serves to show how far natural history illustrations have progressed in the past 130 years. This reprint (Webb & Bower/Holt, Rinehart & Winston; £20, \$40) has a brief introduction by Tony Soper, describing the aims of Morris's work and its impact on the public of his time. The coloured engravings by A.F. Lydon often bear only superficial resemblance to their subjects, the poses being wooden and unnatural; they can have been of little value as guides to the identification of living birds in the field. The worth of a reprint such as this is entirely historical. The book is of no use as a guide for a modern bird watcher but does, through its text, give an insight into the understanding that early ornithologists had of birds.

An artist generally recognized as one of the early masters of bird painting was John Gould, who died in 1873. A beautifully produced volume, John Gould's Birds (A&W Publishers, New York; \$39.95 until December 1981, \$50 thereafter), reproduces a rich selection of his finer work. Maureen Lambourne's introduction provides a biographical sketch of this productive and much-travelled artist. Gould's paintings are essentially decorative and are mostly in the form of vignettes, with the subject posed against foliage or a hint of landscape. Some of his work was remarkably ambitious, such as his painting of a barn swallow feeding her young in flight. His cock pheasant, far from being shown in its customary splendour, is portrayed lying dead in a snare. Gould's scrupulous attention to detail shows, not only in his birds, but also in the plants with which many are framed. Though his pictures are often stilted and contrived by today's standards, Gould's skill and accurate observations make this book a pleasure to look through.

The latest tribute to Archibald Thorburn, who died in 1885 (Thorburn's Landscape by John Southern, published by Elm Tree Books at £12.50), reproduces many of his larger paintings, in which as much attention is devoted to the landscape as to the birds. Most of the paintings are of game birds and are set in moorland, mountains and forest. Thorburn's skilful treatment of light, dark and colour beautifully evokes the chill of autumn or the bleakness of a snow-covered mountain side. Like Gould, Thorburn sometimes attempted a painting more ambitious than a posed group of birds, such as a peregrine killing a mallard in flight or a group of ptarmigan cowering as an unseen golden eagle casts its shadow over them. His rich use of colour and intimate knowledge of the habits of birds make this a fine book.

Following his death in 1979, a number of books have been published that display the work of Charles Tunnicliffe. Robert Gillmor edits and introduces the latest of them, Sketches of Bird Life (Gollancz; £10.95). Most of the book is devoted to Tunnicliffe's field sketches that provided the raw material for his major paintings. An interesting feature of this book is the way that it shows how the artist developed his work from field sketch to finished painting; more examples of this would have been welcome. The sketches, mostly in pencil or watercolour, reveal that Tunnicliffe was not only a supreme artist, but also a fine ethologist. Behavioural interactions between birds are beautifully recorded, both visually and in the annotations that appear on many of the sketches. Tunnicliffe's extreme devotion to his art was remarkable, as shown by his meticulous paintings of dead birds which, together with his field sketches, he used in the creation of his finished paintings.

The work of a new, living artist is presented in Keith Brockie's Wildlife Sketchbook (Dent/Macmillan, New York; £9.50, \$19.95). Working in Scotland, Brockie has carried on the Tunnicliffe tradition, never missing an opportunity to record a scene, a flower, a piece of driftwood or a dead bird, as well as patiently sketching living animals from a hide. Working mostly in pencil, crayon and watercolour, Brockie has the gift of being able to catch the essence of an animal, whether it is a bird, a fish or a rabbit. All that this attractive book lacks are some examples of how the artist might use his sketches to produce more formal paintings.

In sharp contrast, the contemporary American artist Robert Bateman specializes in huge paintings, beautifully

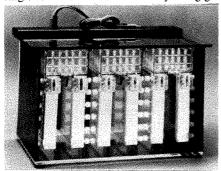


Three redstarts (Phoenicurus phoenicurus phoenicurus) painted by John Gould.

reproduced in The Art of Robert Bateman with text by Ramsay Derry (Viking/Allen Lane; \$40, £20). Bateman's breathtaking pictures are not pretty portraits of animals. His aim is clearly to emphasize the close relationship between an animal and its environment and, in many of his paintings, the animals seem to take second place to the landscape. There is much variety in the scope of his view, from panoramic vistas of Arctic tundra or the East African plains to restricted glimpses up into a tree or through the window of a derelict house. Bateman is an artist of many skills. Not only are his birds and mammals painted with exquisite accuracy of detail and posture, but his use of light and colour perfectly captures the icy cold of the tundra and the heat of the Serengeti plain. These are remarkable paintings that repay long and careful study, and this book stimulates a keen desire to see the original works.

Tim Halliday is a Lecturer in Biology at the Open University, and an author and illustrator of a number of books on natural history including Sexual Strategy (Oxford University Press, 1980) and Vanishing Birds (Penguin, 1980).

Electrophoresis unit. For two-dimensional or multiple one-dimensional electrophoretic separations, Hoefer Scientific Instruments have introduced a new multislab electrophoresis unit. The SE700 has three upper buffer chambers, each containing two 16 cm-long slabs. All six gels are run in identical conditions because all three upper buffer chambers fit into a single lower buffer chamber. By using gel



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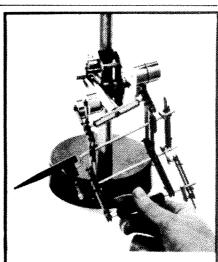
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# UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF GENETICS

# RESEARCH ASSISTANT

position is available, from January 1982, for 2 years, on a esearch project funded by the Zancer Research Campaign, for a graduate or MSc research assistant; xperience with living mice and nouse cytology would be an idvantage but not essential. Salary at ige 23 £5,680.

Further details may be obtained rom Dr Margaret E Wallace to whom applications, with the names of two referees, should be sent to each her preferably by 31st December 1981, Department of Benetics, Downing Street Eambridge CB2 3EH. Tel: 69551. Street,

#### **BIOLOGY TEACHING OVERSEAS**

Positions open in Biological Sciences at American University of Beirut for the academic year 1982-83. Applications are invited for positions in the following specialties: developmental biology, plant biochemistry plant taxonomy/anatomy. PhD required; rank and salary depend on experience and qualifications, but normally appointments are in the rank of Assistant Professor. Usual contract is for three years.

Curriculum vitae and three letters of recommendation should be forwarded to Chairman, Biology Department, American University of Beirut, Beirut, Lebanon. An Affirmative Action/ Equal Opportunity Employer

(NW120)A

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(NW135)A

# **AUSTRALIA** CSIRO **Plant Biologist** (Research Fellow)

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CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,500 employees — 2,700 of whom are research and professional scientists located in divisions and sections throughout Australia.

General: The Division carries out research on problems fundamental to agricultural production. Its Genetic Resources and Biosystematics Section is concerned with increasing taxonomic and evolutionary knowledge of the native Australian flora and with the collection, conservation and utilization of genetic variation in crops and pasture plants.

Duties: Undertake research concerned with the population genetics and utilization of Australian wild relatives of cultivated plants. This will involve team research based on studies of genetic heterogeneity, reproductive biology, disease resistance, applied breeding and testing. The appointee will be required to contribute to one or more projects using relevant techniques which may include experimental crossing, fertility and compatibility testing, cytology, electrophoresis, yield and quality testing.

Qualifications: Applicants should have a PhD degree in an appropriate field or postgraduate research experience of equivalent standard or duration supported by satisfactory evidence of research ability.

Tenure: The appointment is for a fixed term of 3 years. Superannuation benefits available.

Applications: Stating full personal and professional details the names of at least two referees, and quoting reference No. A4695 should reach: The Chief, Division of Plant Industry, CSIRO, PO Box 1600, Canberra City, ACT 2601, Australia by 1 January 1982. (003)A

# MEMORIAL UNIVERSITY OF NEWFOUNDLAND

Canada

FACULTY OF SCIENCE **HEAD OF THE** DEPARTMENT OF PHYSICS

Applications and nominations are invited for the post of Head of the Department of Physics at Memorial University, Applicants should be able to demonstrate ongoing and outstanding achievements in research in a field of physics compatible with the interests and development of the Department. They should also be committed to maintaining and further developing a strong under-graduate and graduate program. This position offers a unique opportunity to interact with new developments in the University and the Province.

The appointment will be at the rank of Full Professor, to take office by 1st September 1982 or such other time as may be mutually agreed. Salary will be negotiable and related to qualifications and experience.

Applications and nominations Applications and nominations should be forwarded, not later than 15 February 1982, to: The Dean of Science, (Dr P J Heald), Faculty of Science, Memorial University of Newfoundland, St John's, Newfoundland, Canada AlB 3X7, from whom further particulars can be obtained. be obtained.

Canadian Government policy requires the statement: "Canadian citizens and/or landed immigrants "Canadian will be given the first consideration from amongst applications received to this first advertisment." (018)A

#### YALE UNIVERSITY

DEPARTMENT OF GEOLOGY AND GEOPHYSICS

Applications are solicited for a faculty position in solid earth geophysics to begin in the academic year 1982-83. Areas of interest to the Department include seismology, exploration geophysics, mechanical and physical properties of rocks and minerals, geomagnetism, and tectonophysics.

Yale University is an equal opportunity/affirmative action employer. and encourages women and members of minority groups to compete for this position.

Curriculum vitae, publications and the names of three or more referees should be sent by 31 December 1981 to Robert B Gordon, Chairman, Department of Geology and Geophysics, PO Box 6666, New Haven CT 06511. (NW134)A

#### ASSISTANT PROFESSOR **PHYSIOLOGY**

The Department of Physiology at the University of Minnesota invites applicants for a tenure-track appointment at the Assistant Professor level beginning about July 1, 1982. Selection will be based primarily on demonstrated research excellence. Applicants must have at least one year of postdoctoral experience and be prepared to carry out independent research.

The research must be in the field of cellular physiology, preferably involving one of the following areas: membrane transport, intracellular ionic concentration measurements, single channel studies, quantitative biology of excitable membranes, membrane reconstitution of transport proteins. The applicant will be expected to participate in the graduate and undergraduate teaching programs of the department.

Send curriculum vitae, including undergraduate and graduate transcripts, a summary of planned research and the names and telephone numbers of three references to: Search Committee, Departmen of Physiology, 6-255 Millard Hall 435 Delaware Street SE, University Minnesota, Minneapolis Minnesota 55455.

The University of Minnesota is a equal opportunity educator and employer and specifically invites ar encourages applications from womeand minorities. (NW120)A

# Director of Regulatory Affairs

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or telephone High Wycombe 21124.

SEARLE

(011)A

# THE INSTITUTE FOR ADVANCED STUDY

will have several openings for nembers in theoretical physics and astrophysics for the academic year 1982-83. The positions are at a post-loctoral or higher level and applicants will be selected on the basis of heir ability to do research in the areas of elementary particles, mathenatical physics, astro-physics, plasma physics, general relativity and tatistical mechanics. Preference is given to candidates who have received their PhD within the last rear or two.

Postdoctoral members frequently collaborate with each other, with aculty members at the Institute or Princeton University, and with esearchers at other institutions.

Appointments are usually for no nore than two years and support is ypically full salary for postdoctorals and half salary for more senior persons. Women and minorities are incouraged to apply.

For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW1006)A

# NATIONAL BIOLOGICAL STANDARDS BOARD Post-Doctoral Scientist

Division of Viral Products require a research scientist to join an active research group working on INTERFERONS. The studies will involve the production of interferon by human T-cel clones and hybridomas, and its characterization by molecular and immunogical methods. The post provides an opportunity for a young scientist to gain experience in a rapidly expanding field of medical importance.

Applicants should have recently completed a PhD degree in a relevant field.

The appointment is for three years and is supported by a grant from the Cancer Research Campaign.

Starting salary will be commensurate with age, qualifications and experience on the scale £6,070 to £9,750 plus £967 London Weighting.

Further details and application form from Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead NW3 6RB. Ref no SG/295. Closing date 21/12/81.

# Re-advertisement NATIONAL BIOLOGICAL STANDARDS BOARD PHARMACOLOGIST/ PHYSIOLOGIST

Division of Hormones have an immediate vacancy for a post-doctoral pharmacologist/physiologist. The work currently involves research on the mechanism of action and methods of detection of pyrogens, and in the characterisation of small pharmacologically-active substances such as gut and neuroactive peptides.

Applicants should have a postdoctoral degree in pharmacology or physiology and have some experience of laboratory pharmacology research.

The salary payable is in accordance with qualifications, age and experience on the MRC Grade II (£7,037 pa — £10,717 pa inclusive).

For further details and an application form please contact the Personnel Officer, NIBSC, Holly Hill, Hampstead, London NW3 6RB. Tel: 01-435 2232. Please quote ref no HO/049. Closing date 21st December 1981.

(9987)A



DEPARTMENT OF CHEMISTRY GRADUATE STUDIES

GRADUATE STUDIES IN CHEMISTRY 1982/83 Academic Year

Applications are invited from candidates with high academic records who wish to pursue further studies towards the MSc and PhD Degrees in Chemistry. Excellent research facilities are available in all areas of modern Chemical Science, ranging from Bio-Inorganic Chemistry to Chemical Physics. Teaching and research assistantships are available for all students in the program. The total minimum stipend is \$10,000 for MSc students, with higher amounts for PhD students and holders of graduate scholarships. Application forms and further particulars may be obtained from: James Trotter, Professor and Acting Head, Department of Chemistry The University of British Columbia, 2036 Main Mall, Vancouver, BC

(NW130)A

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Please write with detailed c.v. to the attention of J.P. Dembour, Personnel Departement, rue du Tilleul 13, B-1320 GENVAL (Belgium).

(W520)A

# UNIVERSITY OF CAPE TOWN Senior Analytical Scientist in Archaeology

Applications are invited for the above post in the Archaeology Department, vacant as of January 1st, 1982.

The appointee will hold the rank of Lecturer in Archaeology with primary responsibility for building and developing a research laboratory for ratio mass spectrometry of carbon, oxygen, nitrogen, and hydrogen isotopes, utilising a VG Micro-mass 602E instrument. Further duties will include participation in the development of research and teaching programmes in science-based archaeology, with emphasis on mass spectrometry, but including other analytical techniques. It is expected that the appointee will be qualified at the Doctoral level (or equivalent) in a natural or physical science, have research interests relevant to archaeology, and have extensive experience with analytical instruments.

The post is a permanent staff position, subject to a 3-year probationary period, but a 3-year contract appointment could also be considered. Appointment will be made, according to qualifications and experience, on the salary scale R10 995 x 675 — R14 370 x 810 — R19 230 per annum. In addition a service bonus of nearly one month's salary is payable

Applicants should submit a full curriculum vitae, stating present salary, age, publications, relevant qualifications and experience, the date on which duty could be assumed and the names and addresses of three referees.

Further information should be obtained from the Head of the Archaeology Department and applications should be sent to the Registrar, (attention: Appointments Office), University of Cape Town, Rondebosch, 7700, South Africa, by whom applications (quoting ref no AC/11) must be received not later than February 15th, 1982 (late applications may be accepted).

The University's policy is not to discriminate in the appointment of staff on the grounds of sex, race or religion. Further information on the implementation of this policy is obtainable from the Registra.

W517)A

# SIMON FRASER UNIVERSITY

Burnaby, British Columbia, Canada

DEPARTMENT OF BIOLOGICAL SCIENCES

# POPULATION DYNAMICS

Applications are invited for a tenure-track position as Assistant Professor in Population Biology, with research interests in the dynamics of interspecific interactions (eg, predator-prey, parasite-host, pathogen-host). The successful applicant will be expected to develop a strong research programme and to teach at both the undergraduate and graduate level, including courses in the Department's Master of Pest Management Programme. Candidates must have a PhD degree, with experience in entomology and/or pest management. Preference will be given to candidates eligible for employment in Canada at the time of application.

The position is available from 1 September 1982. The current salary base for the Assistant Professor rank is \$25,000/year subject to ratification.

Applications should include curriculum vitae, a brief statement of research interests and objectives, and reprints of published research. Applicants should request a confidential assessment of their research and teaching ability from three referees, to be forwarded directly to: Dr K K Nair, Chairman, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6.

Deadline for receipt of applications is 31 December 1981, or until the position is filled. (NW119)A

# THE UNIVERSITY OF ALBERTA

Edmonton, Alberta Canada T6G 2E3

DEPARTMENT OF GEOLOGY

The Geology Department has one permanent faculty position available (subject to funding) July 1, 1982. We invite applications from qualified individuals for appointment at the Assistant or Associate Professor level in any of these areas: Geomor-phology, Mathematical Geology, Engineering Geology, Process Sedimentology and Structural Geology, Preference will be given to those applicants who demonstrate an ability to pursue a vigorous research program applying modern concepts and techniques in solving geological problems. The candidate is expected to teach an undergraduate course in quantitative geomorphology, course(s) in his or her speciality, including if qualified, geostatistics. The position also involves supervising Masters and PhD students. A PhD is required and salary is commensurate with education and experience. Canadian citizens and permanent residents will be given preference.

Interested applicants should sumbit a résumé, publications and names and addresses of three referees to Dr N W Rutter, Chairman, Department of Geology, University of Alberta, Edmonton, Alberta, Canada T6G 2E3. Closing date for applications is February 15, 1982. The University of Alberta is an equal opportunity an employer.

(NW127)A

# DEPARTMENT OF ZOOLOGY University of California, Davis ASSISTANT PROFESSOR

Applications are invited for a tenure-track position at the Assistant Professor level for September 1982. A strong, independent research program is expected; fields of particular interest include, but are not limited to, evolutionary biology, marine or behavioral ecology, and environmental physiology. Preference will be given to candidates able to teach invertebrate zoology at the upper-division level. The appointee may also teach a one — quarter course in introductory biology and will have the opportunity to develop courses and/or seminars in his/her area of expertise.

Persons wishing to apply should send a curriculum vitae, reprints of published work, a description of research program and future plans, a summary of teaching experience and capabilities including evaluations if available, and three letters of recommendation to:

Dr. Arthur M. Shapiro,

Dr. Arthur M. Shapiro,
Chairman, Search Committee,
Department of Zoology,
University of California,
Davis, CA 95616.
Final Date for receipt of applications is February 15, 1982.

Final Date for receipt of applications is *February 15, 1982*. The University of California is an equal opportunity/affirmative action employer. (NW101)A

# MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF MICROBIOLOGY CONTINUING LECTURER/SENIOR LECTURER

The Department conducts courses leading to BSc and BSc (Hons) at the Clayton Campus and to MBBS, BMed Sci at the Monash Medical School, Alfred Hospital. The successful applicant would be expected to teach in appropriate areas of these courses and to supervise graduate students for MSc and PhD. Activities of the Department include molecular biology, genetics, virology, microbial biochemistry, medical microbiology, pathogenesis of infections and immunology. Applicants should possess either MD or FRCPA, or PhD or equivalent qualifications. Experience in medical microbiology or related fields an advantage. Excellent research facilities available and opportunities exist for a suitable appointee to participate in the diagnostic, clinical and research work of the teaching hospital of the University, including the Alfred Hospital and Fairfield Hospital for Infectious Diseases. Annual salary: Lecturer \$A19,821 — \$A26,036; Senior Lecturer \$A26,593 — \$A30,995; Medically qualified Lecturer \$A23,151 — \$A29,367 or Senior Lecturer \$A29,923 — \$A34,325. Superannuation, travel and removal expenses, Outside Studies Programmes and temporary housing assistance. Enquiries about the position may be addressed to Staffing Committee, Department of Microbiology in the University

Applications including Ref No 30912, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Vic. 3168, Australia, with a copy to the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, as soon as possible and not later than 5/2/82. Conditions of appointment can be obtained from the Association. (9994)A

STATENS SERUMINSTITUT (Copenhagen)

# DEPUTY HEAD OF DEPARTMENT

A position is available in the Enterovirus Department from 1st February 1982 or as agreed upon. Responsibilities will include routine functions and basic and applied research. Routine functions comprise the preparation of cell cultures for the production of inactivated polio vaccine (IPV), culture and inactivation of polio virus, carrying out all control tests which are required during production and before release of IPV as an independent vaccine or as a partial product of Di-Te-Pol vaccine. The work also includes testing and evaluation of other virus vaccines.

The department also functions in an advisory capacity in questions relating to vaccines and vaccination and postgraduate education. In the future this section will be responsible, in collaboration with other production departments, for the entire production and control of vaccines at the Institute and will probably be incorporated with one or severel departments in a production section, at a later date.

Candidates should be conversant with production and control of biological products. Experience in microbiological, biochemical and/or immunological methods would be an advantage.

The ideal candidate is a person of proven research ability with a flair for leadership.

The position is permanent, salary 271.500 Danish kroner. The work will be carried out at Statens Seruminstitut, but the appointment is under the Ministry of the Interior.

Applications should be addressed to Her Majesty the Queen and five copies should be sent to the Directors, Statens Seruminstitut, Amager Boulevard 80, DK-2300 Copenhagen S, by 4pm 8th January 1982.

(W522)A

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Division of Computing Research
Canberra Act

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,500 employees — 2,700 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

A scientist with biological and computer modelling experience is required to work in the Division's simulation section which is concerned with modelling complex systems which may have physical, biological and economic aspects. Projects of biological interest include: developmental biology, biotechnology, insect and parasite control, plant and animal growth and aquatic and terrestrial ecosystems. Such projects are carried out in close collaboration with staff of other Divisions of CSIRO.

The section is multi-disciplinary, with expertise in computing, systems theory and the physical sciences and considerable experience in biological modelling. Research interests of the simulation section also include programming, numerical methods and all aspects of the methodology of modelling.

The Division's research staff are located in close proximity to several of the major biological Divisions of CSIRO and to the Research Schools of Sydney University and the Australian National University. The appointee will have opportunities for close contact with biological scientists and modelling groups throughout Australia.

The Divison has branches in most Australian cities and provides to other Divisions a high-level consulting service covering most phases of advanced computer use. Fields in which other sections of the Division are working include operating systems, data-base management, image processing, graphics, micro-electronics and numerical taxonomy.

The Division has a Control Data Cyber 76 computer as the main component of a very large multi host network known as CSIRONET. This provides a powerful tool for research and development, and enables scientists in CSIRO laboratories throughout Australia to use both central and distributed facilities for either batch or interactive computing. It also allows sharing of data and cooperative development of programs.

**Duties:** (1) Within a group of diverse computing and modelling specialists, to collaborate extensively with biological groups applying simulation modelling to practical problems.

(2) To develop further the computing and theoretical techniques needed for the simulation and dynamic analysis of biological and other complex systems.

Qualifications: A PhD degree in an appropriate biological field or equivalent qualifications together with demonstrable research ability. Considerable computational skill and ability to develop computer models is essential.

Tenure: 3 years with superannuation.

Applications: Stating full personal and professional details, the names of at least two referees and quoting reference No A1895, should reach: The Chief, Division of Computing Research, CSIRO, PO Box 1800, CANBERRA CITY, ACT 2601 AUSTRALIA by 1 January 1982.

# AUTONOMIC NEUROPHYSIOLOGIST RESEARCH ASSOCIATE

to serve as co-investigator of NASA-funded experiment to be conducted on astronauts in space shuttle. Applicant must have doctoral degree and be an independent, established scientist with a broad understanding of autonomic cardiovascular physiology, and peculiar interest and skill in electrophysiology and/or central autonomic neuroanatomy.

Send curriculum vitae and salary requirements to Dr Dwain L. Eckberg, Cardiovascular Physiology, V.A.Medical Center, Richmond, Virginia 23249. (NW122)A

# **Jirector**

# **MRC Trauma Unit: Manchester University**

The Medical Research Council invites applications for the post of full-time Director of the MRC Trauma Unit, Manchester. The present Director, Professor H B Stoner, retires in September 1984: the successful candidate would be encouraged, subject to mutually acceptable arrangements, to take up appointment earlier, as Director-designate.

The present work of the Unit aims to lead to improvements in the care of the injured. The research programmes are a combination of clinical, laboratory and experimental studies. The Council wishes the emphasis on research in trauma to be maintained; it will however review the title and programme of the Unit in the light of the research interests of the new Director.

The University of Manchester will consider conferring on a suitable appointee an honorary Professorship in

The University of Manchester will consider conferring on a suitable appointee an honorary Professorship in an appropriate discipline.

The Unit is housed in the Medical School (Stopford Building) of the University of Manchester and has clinical laboratory accommodation, with facilities for the study of acutely injured patients, at the Hope Hospital, Salford, where a new NHS Accident and Emergency Department and an Intensive Care Unit (10 beds) will be commissioned in 1984.

rent and an intensive Care unit (10 beds) will be commissioned in 1984.

The eventual scale and composition of the staffing of the Unit will be a matter for discussion between the new Director and the Council.

Applicants should be medically qualified and eli-

Applicants should be medically qualified and eligible for honorary consultant status in an appropriate speciality. It is expected that the person appointed would be of high scientific standing with an established reputation as an original scientific investigator and a capacity to direct both the clinical and the basic aspects of the Unit's programme. It is considered essential for the success of the venture that the present high level of clinical collaboration with the NHS staff at the Hope Hospital should be maintained and an ability to foster this relationship will be a key quality sought in the successful applicant.

An honorary clinical contract with the Salford AHA (1) (6/11ths) at consultant level in an appropriate speciality will be offered to the successful candidate. The appointment carries a salary on the MRC clinical scale, which is equivalent to NHS consultant grade.

Further information may be obtained from Mr R

Further information may be obtained from Mr R Bush, MRC Headquariers Office, 20 Park Crescent, London W1N 4AL Tel 01-636 5422



Ext 353. Applications, including the names of three referees, should be submitted to the Secretary of the Council at the same address, not later than 22 Medical Research Council January 1982.

# AGRICULTURAL RESEARCH COUNCIL POULTRY RESEARCH CENTRE Roslin, Midlothian EH25 9PS **SCIENTIFIC OFFICER**

A Scientific Officer is required in the Ethology Department to assist with research on poultry behaviour and welfare. This research project seeks to identify and correlate behavioural and physiological parameters which may be indices of stress. The successful candidate will be expected to participate fully in all aspects of the work. Duties will include implanting telemetry devices, observing and recording behaviour patters of birds, collaborating over data collection and sorting, using a PDP11 computer, day-to-day supervision of experimental birds and drafting reports on experiments and papers for publication. The Officer will report directly to the Head of the Ethology Department and be responsible for supervising an A.S.O.

Qualifications: A pass degree (or equivalent) in Biology, Zoology, Psychology, Agricultural Science, or related discipline. A good general knowledge of animal behaviour and physiology is required. Some knowledge of statistics and computer programming would be an advantage.

Salary: In scale £5,176 - £6,964. Non-contributory pension scheme. 20 days annual leave.

Application forms are available from Mrs Hunter, Assistant Secretary, at the above address. Completed forms must be returned to her by the 11th December 1981.

# **CSIRO**

# **AUSTRALIA**

# **Assistant Chief** (Geneticist)

\$A29,638 - \$A38,921

# **Division of Animal Production Prospect, New South Wales**

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximatley 7,500 employees 2,700 of whom are research and professional scientists - located in divisions and sections throughout Australia.

Field: Genetics

The Division of Animal Production, with Headquarters at Prospect, near Sydney, New South Wales, conducts research aimed at understanding the genetic, nutritional and physiological basis of animal functions with the aim of improving the efficiency of livestock

Duties: The appointee will assist the Chief of the Division in the guidance and coordination of a research group with interests ranging from basic research in molecular and developmental genetics to animal breeding in poultry, sheep and cattle. Future basic research in the genetics program will be broadly directed at the application of new genetic technologies to animal improvement, and the successful applicant will be expected to provide scientific leadership in this area. In addition, the appointee will liaise with scientists in the Division who are studying skin biology, nutrition and reproduction, and to aid in the development of inter-disciplinary research programs to improve animal production.

Qualifications: Applicants should have a PhD degree or equivalent, a substantial record of research achievement in genetics and the ability to lead a research team. An interest in the application of molecular and cellular biology and quantitative genetics to improving animal production is essential.

Tenure: This is an indefinite appointment which carries Australian Government Superannuation benefits. However, the role of Assistant Chief is offered for an expected term of seven years.

Note: An outstanding applicant may be offered appointment at a higher level

Applications: Stating full personal and professional details, the name of at least two referees, and quoting reference No A0905 should reach: The Chief, Division of Animal Production, CSIRO BLACKTOWN, NSW 2148 AUSTRALIA by 1 January 1981. (006)A

#### MONASH UNIVERSITY Melbourne, Australia

DEPARTMENT OF MATHEMATICS FIXED TERM (5 YEARS) LECTURER IN APPLIED MATHEMATICS

Research groups are active in astrophysics and geophysical fluid dynamics, and preference will be given to applicants whose research relates to the gfd group involving basic fluid dynamics and its applications to dynamical meteorology and oceanography, and geophysical data analysis.

Salary: \$A19,821 to \$A26,037 pa. Superannuation, travel and removal expenses, temporary housing assistance and repatriation.

Enquiries to Professor BR Morton Enquiries to Professor B R Morton in the University. Applications including Ref no 40112, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Vic. 3168, Australia, with a copy to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1 OPF, by 31/12/81. Conditions of appointment can be obtained from the Association. (9990)A the Association.

# UNIVERSITY OF DUNDEE DEPARTMENT OF BIOCHEMISTRY **MOLECULAR GENETICS** LABORATORY RESEARCH ASSISTANT

Applications are invited from graduates with good honours degrees in Biochemistry or related subjects to work on the sequence dependence of DNA structure, with an emphasis on the construction and cloning of novel DNA species by recombinant DNA techniques. Some experience of nucleic acid manipulation will be an advantage. The successful candidate will work with Dr D M J Lilley in an expanding laboratory containing young and enthusiastic researchers, mostly involved in cloning studies.

The appointment, which is MRC funded, is available immediately at an initial salary of up to £6,070 and is expected to last for 3 years. There is a possibility of registration for higher

Applications containing full career details and the names of two referees should be sent by 24th December 1981 to The Personnel Officer, The University, Dundee DD1 4HN and should quote Ref: EST/60/81J.

# Fermentation Microbiology

A microbial biochemist/microbiologist is required to join an established group involved in various aspects of the biochemistry of yeast and fermentation processes. The person appointed will work for Britain's largest brewing group, in the research department, located at Burton-on-Trent, Staffordshire.

He/she will contribute to our understanding of yeast metabolism and fermentation technology. Applicants must have a Ph.D. degree and possibly post-doctoral experience in a relevant field of biochemistry or microbiology and be capable of self-motivated, high quality research.

The ability to innovate and achieve rapid progress towards defined objectives are essential attributes. Encouragement will be given to maintain and extend liaison with academic laboratories and to publish results where appropriate.

Salary will be commensurate with age and experience. The Company operates a generous life assurance and pension scheme and provides comprehensive recreational facilities.

Detailed applications, including a curriculum vitae and the names of two referees should be sent as soon as possible to:-

Recruitment & Training Manager, Bass Limited, 137 High Street, Burton-on-Trent, Staffordshire DE14 1JZ.

# Bass Limited

(014)A

# MEMORIAL UNIVERSITY OF NEW FOUNDLAND DEPARTMENT OF BIOCHEMISTRY PROSPECTIVE POSTDOCTORAL TRAINEES

graduate students and research fellows are encouraged to apply for research positions in the area of environmental and marine biochemistry, antifreeze proteins, membrane glycoproteins, intermediary metabolism, physical chemistry of lipids and proteins, experimental and community nutrition, microbiology, food sciences, food processing and food engineering. Grant-funded research in these areas is conducted in well-equipped laboratories by highly motivated research faculty. Salaries of postdoctoral and research fellows, other benefits and starting dates are negotiable. Fellowships of graduate students may be supplemented from grants and teaching assistantships.

Apply with vitae and two letters of recommendation to Dr S Mookerjea, Head, Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X7, Canada. (9979)A

#### **CELL BIOLOGIST**

The CV Whitney Laboratory, a research center of the University of Florida, invites applications for a full time, tenure-earning research position at the assistant or associate professor level. The successful applicant will be expected to develop a strong independent research program and to interact with existing projects in nuerophisiology, neurochemistry, cellular immunology, biochemistry and membrane transport. All applicants with good ideas on fundamental questions will be seriously considered, but preference be given to applicants with training or experience in immunology who would be interested in taking advantage of marine organisms to study problems in neurobiology, endocrinology, comparative im-munology, cell-cell interaction or fertilization.

Send letter stating current and future research interests, curriculum vitae and names of four references by December 31, 1981 to: Search Committee, CV Whitney Laboratory, University of Florida, Rt 1, Box 121, St Augustine, FL 32084. An Equal Opportunity/Affirmative Action Employer. (NW128)A





Canada Service

Research Scientists

Salary: \$23,225 - \$43,200 Ref. No.: 81-NCRSO-DFO-7, 8 and 9

Department of Fisheries and Oceans Ocean Science and Surveys Dartmouth, Nova Scotia

The Bedford Institute of Oceanography, Dartmouth, Nova Scotia, requires two scientists to participate in marine environmental quality research and a third scientist to conduct research into the ecology and life history of fish.

#### MARINE PHYSIOLOGICAL BIOCHEMIST

#### 81-NCRSO-DFO-7

Research experience in the study of biochemical and physiological pathways and reservoirs of metal or radio-nuclide accumulation in marine biota is required for this position. Experience is also required in the handling and determination of radioisotopic tracers and ocean sampling equipment.

#### **BENTHIC MICROBIAL ECOLOGIST**

#### 81-NCRSO-DFO-8

For this position, research experience in the study of benthic microbial ecology is required. Experience in the handling and determination of radioisotopic tracers and conducting in situ experiments at sea is also required.

# FISHERIES ECOLOGIST

#### 81-NCRSO-DFO-9

To successfully meet the requirements of this position, experience is required in the planning and directing of laboratory and field programs on the ecology of aquatic production. Also, a demonstrated capacity in statistical and mathematical analysis of ecological systems is required.

#### Qualifications

All three positions require a doctorate or a lesser degree with research experience and productivity equivalent to that of a doctorate.

#### Language requirements

Knowledge of the English language is essential.

Clearance Nos.: 111-211-006

111-211-005

111-258-015

Additional job information is available by writing to the address below:

Toute information relative à ce concours est disponible en français et peut être obtenue en écrivant à l'adresse suivante:

#### How to apply

Send your application form and/or résumé to:

Brian Beyer

National Capital Region Staffing Office Public Service Commission of Canada 300 Laurier Avenue West

Ottawa, Ontario K1A 0M7 Tel. (613) 593-5331 Ext. 497

Closing date: January 29, 1982

(NW117)A

Please quote the applicable reference number at all times.

Canada'



# Greenford, Middx.

c. £10,000-£13,000

required to work on the production of secondary metabolites from fungi, natural and genetically-engineered bacteria and Streptomycetes. We have recently completed a new plant which has shake-flask, 5, 50 and 500 litre fermenters and is fully instrumented and data logged, with extensive on-line and off-line data-handling facilities, including graphics. Your responsibility is to establish the production of new natural and engineered fermentation products using batch and continuous culture. The Biotechnology Department is also concerned with enzymology and both plant and animal cell culture.

You will have several years' post-doctoral experience in microbial physiology and will view our vacancy as a unique opportunity to apply the most modern techniques, in association with genetic engineers, to making a broad spectrum of fermentation products.

The Company offers excellent conditions of employment, including a non-contributory pension scheme, bonus schemes and an active sports and social club. Relocation assistance will be given if appropriate.

Please write or telephone for an application form to: Miss E. M. Butler, Personnel Department, Glaxo Group Research Ltd., Greenford Road, Greenford, Middlesex UB6 0HE. Tel: 01-422 3434, ext. 2707, quoting ref. AQ.399.

GlaxO Group Research Ltd.

(019)A

# CSIRO AUSTRALIA **Biochemist** (Research Fellow)

\$A19,662 - \$A28,564 pa **Division of Plant Industry** Canberra Act

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,500 employees - 2,700 of whom are research and professional scientists - located in divisions and sections throughout

General: The Division conducts pure and applied research in genetics, plant breeding, genetic resources, biochemistry, microbiology, plant nutrition, ecology, crop adaptation, soil fertility and plant introduction.

The position is in the Biochemistry Section where current research includes studies on chloroplasts membranes, protein and nucleic acid metabolism in chloroplasts, photosynthetic carbon matabolism, the biochemistry and physiology of seed proteins, and haemoproteins involved in nitrogen fixation.

Duties: To undertake research on the biochemistry of C4 photosynthesis. This will be part of a project which presently includes studies on enzyme regulation, metabolism of isolated cells and organelles, and metabolite transport processes

Qualifications: Applicants should have a PhD degree or equivalent in an appropriate field. Previous experience in the field of photosynthesis biochemistry would be an advantage

Tenure: The position is available for three years with the possibility of extension up to two years beyond this period if mutually desired. Superannuation benefits available.

Applications: Stating full personal and professional details, the names of at least two referees and quoting reference No A4735 should reach: The Chief, Division of Plant Industry, CSIRO, PO Box 1600, CANBERRA CITY, ACT 2601, AUSTRALIA by 1 January 1982.

# **ROYAL FREE HOSPITAL** SCHOOL OF MEDICINE (University of London)

DEPARTMENT OF BIOCHEMISTRY

#### AND CHEMISTRY **POSTDOCTORAL** RESEARCH ASSISTANT

Physicist or physical chemist required to join research group working on dynamics of biomembranes. Experience of optical methods and laser techniques would be an advantage. Post is tenable for 2 years, supported by the SERC.

Salary on Range 1A: £7,037 £11,542 (inclusive). Applications (cv and names and addresses of two referees) should be sent to the School Secretary, RFHSM, Rowland Hill Street, London NW3 2PF as soon as possible. Please quote ref: SERC/B.

### MANCHESTER AREA **HEALTH AUTHORITY** (TEACHING)

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE

#### RESEARCH STUDENT

Applications are invited from graduates with a good honours degree in a biological subject for a three year research studentship concerned with neuroectodermal tumours of children.

The project will involve im-munological and electron microscopic techniques.

Applications including curriculum vitae and the names of two referees should be sent to the Hospital Administrator, Christie Hospital & Holt Radium Institute, Wilmslow Road, Manchester 20. (9974)A

#### POST-DOCTORAL FELLOW

Post-Doctoral position in Virology to study the genetics of influenza viruses. Project includes 1) molecular cloning of influenza virus genes, sequencing and expression of cloned genes, and 2) analysis of the biological function of individual genes, pathogenicity factors, analysis of epidemiologically important influenza virus strains. Position now available. Please submit cv to Dr Peter Palese. The Mount Sinai School of Medicine, One Gustave L Levy Place, New York, NY 10029. An Equal Opportunity employer.
(NW123)A

### THE NATIONAL HOSPITALS FOR NERVOUS DISEASES Oueen Square,

# London WC1N 3BG RESEARCH ASSISTANT OR SRN

For six months starting from 1st January 1982 to assist with Physiological studies on patients with neurological disorders. You must have a sympathetic approach to patients and previous experience in the neurological field would be useful although not essential. Five-day week (Monday to Friday).

Salary Scale is dependent on experience and educational qualifications.

Application forms obtainable from and returnable to: The at the above Personnel officer address, Tel: 01-837 3611 ext 65.

Closing Date 16th December 1981. (9975)A

# Synthetic Organic and Physical Organic Chemists

We are planning the recruitment of top quality organic chemists for the period 1982-1984, and require innovative and resourceful scientists who will play an important role in drug discovery, as part of a multidisciplinary team. Current projects cover most major disease areas and provide the organic chemist with the challenge of design and synthesis of drug molecules and of understanding the way they interact with biological systems.

We are interested in making contact with candidates in the following categories:

- Synthetic organic and physical organic chemists under 30 with a Ph.D. and post doctoral experience who are seeking a position in 1982.
- Synthetic organic and physical organic chemists under 30 who have recently gained or are completing their Ph.Ds in

1982 and who intend to undertake postdoctoral research before seeking employment in 1983-84.

The Division's research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Salaries and conditions of employment will reflect the importance of these posts and relocation assistance will be provided.

Applications in writing including a detailed C.V. and quoting Ref. No. I.C.P. 663 should be addressed to:



Mr. R. Yates, Personnel Officer, Imperial Chemical Industries P.L.C. Pharmaceuticals Division, Mereside Research Laboratories, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

UNIVERSITY OF WESTERN ONTARIO

UNIVERSITY OF WESTERN ONTARIO

Cancer Research Laboratory POSTDOCTORAL FELLOWS

Staff includes Drs. G. Chaconas, K. Ebisuzaki, E. Faust, D. Fujita and

B. Milavetz. Areas of research include DNA replication, poly
(ADP)ribose metabolism, tumour virology, control of mammalian
cell cycle, and cloning of mammalian genes with particular
reference to cancer. Submit curriculum vitae and letters of
reference to Dr. David Denhardt, Cancer Research Laboratory,
University of Western Ontario, London N6A 5B7 Canada.

(NW102)A

# BRF

# BIOTECHNOLOGIST

The Brewing Research Foundation requires a Biotechnologist with research and development experience to lead, and another to join, a small team concerned with the process engineering, inovation and equipment development relevant to raw material utilization, malting, work production, fermentation, separation processes, by - product formulation, waste treatment energy usage and general biotechnology.

The foundation is the corporate research centre of the brewing industry and is located in pleasant surroundings at Nutfield in Surrey. The team is expected to undertake collaborative projects with individual companies and to facilitate the implement action of new technology. Previous knowledge of the industry is not essential. Many previous foundation staff have moved to senior positions within operating companies following a period of successful research and development at

Conditions of employment are those to be expected of a responsible employer: pensions are under USS. The maximum salary for the senior post will be £14,026 and for the second post £8,543: the initial salaries will depend on age, qualifications and

Informal enquiries are encouraged, otherwise write enclosing a C.V. to Alan Clapp, The Brewing Research Foundation, Lyttel Hall, Nutfield, Redhill, Surrey RH1 4HY. Telephone Nutfield Ridge (Std. 073782) 2272 quoting reference BI. (017)A

POSTDOCTORAL Positions funded for 2 years are available immediately to study phospholipid metabolism in the cardiovascular system. research will focus on the isolation, characterization, and regulation of cardiovascular phospholipase A<sub>2</sub>. Salary: NIH guidelines. Send résumé and three letter of reference to: Dr Richard C Franson, Department of Biophysics, Box 694, Medical College of Virginia, Richmond, Va. 23298. An Equal Opportunity/Affirmative Action Employer. (NW125)A

# PROFESSOR AND CHAIRMAN SOIL SCIENCE DEPARTMENT

University of Florida, Gainesville, Florida. Address inquiries to: Dr D H Hubbell, Chairman, Search and Screen Committee, Soil Science Department, 2169 McCarty Hall, University of Florida, Gainesville, FL 32611. Applications accepted the ough February 15, 1982. An email through February 15, 1982. An equal opportunity/affirmative action (NW126)A employer.

**Royal Melbourne Institute of Technology** 

# Lecturer in Mycology **Department of Applied Biology**

A challenging position is available for an individual to be responsible for establishing Mycology electives for the Applied Biology and Medical Laboratory Science courses in the Department of Applied Biology.

Applicants should possess an appropriate teriary qualification and with experience in botany, microbiology or medical laboratory science. A higher degree and teaching experience are

The position will be offered on a tenured basis. Salary within the range \$A19,821 — \$A26,037 p.a. Further information on this position may be obtained from Dr R. Borland - (03) 341 2110.

A position description is available from Staff Branch, RMIT, PO Box 2476V, GPO Melbourne, 3001 Australia. Applications quoting ref. no. 122-16-AN to the Staff Officer by 15th January, 1982.





# Tree Fruit Breeder

Salary: \$23,225 - \$43,200 Ref. No.: 81-NCRSO-AGR-17

Agriculture Canada Research Harrow, Ontario

The Harrow Research Station, Harrow, Ontario, requires a scientist to conduct research on pear breeding and genetics. Studies will also be conducted on rootstock research and tree fruit breeding.

#### Qualifications

Applicants must possess a doctorate degree or a lesser degree with research experience and productivity equivalent to that of a doctorate. Research experience in pomology and plant physiology is required in order to qualify for this position.

#### Language requirements

Knowledge of the English language is essential.

Clearance No.: 401-265-001

Additional job information is available by writing to the address below:

Toute information relative à ce concours est disponible en français et peut être obtenue en écrivant à l'adresse suivante:

How to apply
Send your application form and/or résumé to:
Brian Beyer
National Capital Region Staffing Office
Public Service Commission of Canada

300 Laurier Avenue West Ottawa, Ontario K1A 0M7 (613) 593-5331 Ext. 497

Closing date: February 28,1982

(NW118)A

Please quote the applicable reference number at all times.



# JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

### RESIDENT SCIENTIST (TOXICOLOGY)

Indonesia

A co-operative development project is being undertaken between the Animal Diseases Research Institute, Bogor, Indonesia (Balai Penelitian Penyakit Hewan) and James Cook University, supported by the Australian Development Assistance Burean.

The project group will develop training programs for Indonesian graduate and technical staff engaged in diagnostic and research duties within the Institute. It is expected that collaborative work will evolve with other institutes in the Bogor area and with regional diagnostic laboratories throughout Indonesia.

Applications are sought for the position of Resident Scientist (Toxicology). The appointee will lead the toxicology section within the Institute and will have responsibility for the training of counterpart Indonesian staff in field and laboratory methods of identifying toxic materials in plants and foodstuffs. In addition, the appointee will be expected to take part in extension activities and develop applied research programs.

Applicants must have a higher degree and substantial experience in research and training, especially at the graduate level. Extensive experience is required in chemical and/or pathological aspects of toxicology in animals, preferably under field conditions. Previous work with mycotoxins would be an advantage. An appreciation of areas of priority and appropriate technology in a developing country is essential and previous work in the tropics is desirable.

The appointment will initially be for two years. The appointee will be expected to take up duty in Indonesia in June 1982 or as soon as possible thereafter and this will be preceded by a 2-3 month period of induction and language training in Townsville, Queensland.

Appointment will be within the salary ranges \$A19821 — \$A26037 per annum or \$A26593 — \$A30995 per annum with the commencing salary being determined on the basis of qualifications and experience. Overseas allowances, assistance with children's educational expenses and assistance with removal and travel expenses will apply as appropriate.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 29 January 1982. (9991)A

POSTDOCTORAL Research Position available to study the role of folyl polyglutamates in tumor cell proliferation. Experience in enzymology and protein chemistry helpful. Send curriculum vitae and names of three references to: Dr D G Priest, Department of Biochemistry, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425. (NW124)A



## UNIVERSITY COLLEGE GALWAY, IRELAND RESEARCH ASSISTANT

Applications are invited from Graduates in Physiology, Biochemistry and allied subjects to study the extra-renal production of glutamine in metabolic acidosis. Experience in substrate and enzyme assays is desirable, but not essential. The research is supported by the

Medical Research Council of Ireland.
Applications including Curriculum
Vitae and referees should be sent to:
Professor D J O'Donovan,
Physiology Department,
University College, Galway,
Ireland, not later than 20 December,
1981. (002)A

# UNIVERSITY OF ABERDEEN DEPARTMENT OF BOTANY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for this NERC-funded post tenable for 3 years from January 1982 to work with Dr I Alexander on patterns of release and transformation of nitrogen in forest humus and its utilisation by ectomycorrhizas. Applicants should have relevant experience in one or more of the following fields; root development, ectomycorrhizas, estimation of fungal biomass or activity, lysimetry, nitrogen transformations in soil.

Salary within Scale 1A as appropriate.

Further particulars from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by 23 December 1981. (9985)A

# INSTITUTE OF CANCER RESEARCH

CHEMICAL CARCINOGENESIS DIVISION

Applications are invited for a

# POSTDOCTORAL RESEARCH POST

at the Institute's laboratories at Pollards Wood, near Amersham, Bucks. The post, funded by the MRC/CRC, is available immediately to work with a group concerned with the molecular basis of mutations induced by chemical carcinogens.

Applicants should preferably have research experience in molecular biology, recombinant DNA technology or a related field. Starting salary in the range £6,880 to £8,515 p.a.

A brief CV (in duplicate) containing an indication of postgraduate research experience should be sent, with the names of two referees, to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref: 300/G/11.

Informal enquiries may be made to Dr P Brookes, telephone 02404-2326. (9993)A

# The University of Arizona Seeks a Dean of the Faculty of Science

The University of Arizona is creating a new College of Arts and Sciences to be composed of the Faculties of Fine Arts, Humanities, Sciences, Social and Behavioral Sciences. The Faculty of Sciences will consist of about twelve departments including mathematics, the physical, and biological sciences in a growing series of a major programs on our campus. The administration recognizes the scientific achievements of its faculty and now desires to enhance both the cohesion and leadership in these disciplines by means of the new organization.

The University is seeking candidates for the Dean of Faculty of Science who have the highest qualifications, both in academic and administrative accomplishments. The appointment will also include a professorship with tenure. The Dean will be responsible for academic, administrative and budgetary activities within his faculty and will report to the Provost of the College of Arts and Sciences. All inquiries, applications, and nominations should be sent to the Office of the Executive Vice President no later than February 1, 1982

A. B. Weaver, Executive Vice President, University of Arizona, Administration 512, Tucson, Arizona 85721.

Equal Opportunity/Affirmative Action Employer. (NW108)A

#### MEDICA **RESEARCH COUNCIL** MAMMALIAN **DEVELOPMENT UNIT**

Applications are invited for a

#### SHORT-TERM NON-CLINICAL SCIENTIFIC POST

in this MRC unit, tenable for 3 to 5 years, to study early mammalian development, Candidates of immediately post-doctoral or equivalent status wil be preferred; molecular or bio-chemical experience will be particularly welcome.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information and an application form may be obtained from The Secretary MRC Mammalian Development Unit Wolfson House, 4 Stephenson Way London NW1 2HE, with whom appli cations, including a full CV and the names of two professional referees should be lodged by December 31 1981. (9920)A



UNIVERSITY OF DUBLIN **Trinity College** 

# **LECTURER** IN BOTANY

Applications are invited for the above post in the Department of Botany, Trinity College, Dublin. The post will be tenable from 1st October 1982.

Preference will be given to applicants with interests in electron microscopy and plant physiology. Salary Scale: £7,006 — £14,557 per annum.

Appointment will be made within the salary range £7,006 - £8,994 p.a.

Application forms and further particulars may be obtained from:

The Establishment Officer, Staff Office. Trinity College, Dublin 2.

Telephone 772941 ext 1775.

The closing date for receipt of applications will be Monday, 21st (9976)A December, 1981.

# MICROBIOLOGY/ **IMMUNOCHEMISTRY**

A Research Fellow is required to join a team investigating the microbiological aspects of dental caries at the Royal College of Surgeons laboratories in Downe, near Orpington, Kent. Much of the work is concerned with the biochemical and immunochemical characterisation of Streptococcus mutans and a wide range of bacteriological, biochemical and immunological techniques is in use. The position would suit a recent PhD in microbiology/biochemistry or a good honours graduate with relevant experience.

The initial appointment will be for 3 years. Salary on the University of London Research and Analogous Staff scale 1B (£6,252 to £8,667) according to experience.

Please apply by sending curriculum vitae to the Personnel Officer, Royal College of Surgeons of England, 35-43 Lincoln's Inn Fields, London WC2A

#### **AMGUEDDFA** GENEDLAETHOL CYMRU NATIONAL MUSEUM OF WALES

DEPARTMENT OF GEOLOGY

Applications are invited for the post of **ASSISTANT** 

(MUSEUM GROUP F)

in the Department of Geology

Applicants should possess a degree in Geology, preferably with a particular interest in mineralogy/petrology; the post will involve a wide range of museological/technical duties

The person appointed will be on salary scale £5,589 - £7,682 with contributory pension rights.

Annual leave entitlement 20 days plus 11 privilege days.

Further particulars of the post may be obtained from: The Secretary, National Museum of Wales, Cathays Park, Cardiff CF1 3NP to whom applications should be submitted not than Friday, January 15t (9983)A 15th,

# CSIRO

# **AUSTRALIA**

# Division of Soils Adelaide SA

# **Chief of Division**

Applications are invited for the position of Chief, Division of Soils, from scientists who have an outstanding record of personal research achievements and leadership in soil science, or other appropriate fields. The position will become vacant in December, 1982, on the expiry of the term of appointment of Dr. A.E. Martin as Chief. If able to join the Division before this date, the succesful applicant will be appointed as Chief Designate and have the opportunity to commence planning the future course of the Division.

CSIRO is Australia's largest and most comprehensive research

organization, having approximately 7,500 employees of whom 2,700 are research and professional scientists. Its broad charter covers research into problems of primary and secondary industries, and also such fields of community interest as human nutrition, the environment and the development and use of natural resources. The Organization's research activities are grouped into five Institutes, and the Division of Soils is one of the Divisions in the Institute of Biological Resources. The other constituent Divisions of the Institute are Entomology, Forest Research Horticultural Research, Irrigation Research, Land Resources Management, Land Use Research, Plant Industry, Tropical Crops and Pastures and Wildlife Research.

The Division of Soils has its headquarters at Adelaide, and also operates research laboratories at Brisbane and Townsville (Qld), Canberra (ACT) and Hobart (TAS). The total staff of the Division is 205, which includes 100 professional scientists.

The objectives of the Division are to conduct research into soil science, including the physics, chemistry and biology of soils, together with the integrative disciplines of pedology and geomorphology and to develop the principles for its application in both dryland and irrigated agriculture, forestry, hydrology, engineering and conservation.

The Chief of Division is responsible to the Institute Director for the leadership, development, scientific direction and integration of the Division's research programs, and is encouraged to promote active collaboration with other Divisions, industry and research bodies working in associated areas.

The salary for the position is negotiable, but will not be less than

\$A43,988 per annum

Appointment to CSIRO is for an indefinite period and carries
Commonwealth Government superannuation privileges, subject to normal conditions. The position of Chief is offered for a negotiable term of the order of seven years, with subsequent options for a further term, if mutually desired, or for a senior position in the

Organization.
The Executive of CSIRO has appointed a Search Committee to seek suitable candidates for this position, and the Chairman of the Committee is Mr. M.V. Tracey, OA, FTS, Director of the Institute of Biological Resources. Mr. Tracey would be pleased to discuss the position with potential applicants and to recieve advice concerning this appointment from people with a particular interest in it. He may be contacted at the address below.

Applications, stating full personal and professional details, and quoting reference No. A5187, should be submitted to: Mr. M.V. Tracey, OA, FTS, Director, Institute of Biological Resources, CSIRO, PO Box 225, Dickson, ACT 2602, Australia by 1 January 1982.



# **UNIVERSITY OF ROCHESTER DEPARTMENT OF PHYSIOLOGY**

Postdoctoral Research Associates (Fully funded positions available)

#### Research opportunities in:

- The biophysics and electrophysiology of excitable membranes: nerve and heart muscle. Voltage clamp measurements of ionic currents, techniques of spectral analysis and optical studies.
- 2. Neural control of the circulation: roles of renin-angiotensin system.

#### For information write:

Dr. Ted Begenisich, Dr. Robert S. Kass, or Dr. Martha L. Blair Department of Physiology Box 642 University of Rochester, Medical Center

Rochester, N.Y. 14642.

(NW136)A

La Société L'OREAL recherche:

# CYTOGENETICIEN

titulaire d'un DOCTORAT D'ETAT ou d'un PhD

pour mener à bein des études de mutagénèse, superviser leur exécution et élaborer des programmes de recherche.

> Lieu de travail: Aulnay/Bois (NE de PARIS) Le candidat devra être bilingue anglais/français.

> > Envoyer CV + photo à: L'OREAL — M. JEGO 1, avenue de Saint Germain 93601 AULNAY SOUS BOIS FRANCE.

> > > (W515)A

Opportunities in the Central Laser Division for

# A Laser Scientist and a Theoretical Physicist

vacancy exists for a Laser Scientist in the Glass Laser Group of the Laser Division. The Laser is used for research by University of the Laser Division. The Laser is used for research by Oniversity and other groups primarily for the study of laser-plasma interactions and laser driven compression by providing seven beam lines at two wavelengths, a range of pulse durations and maximum peak power approaching one Tw per beam. Applicants should hold a good honours degree and have several years experience in the use of high power lasers in plasma physics or a similar research field. A thorough knowledge of laser physics and experience in the operation of high power glass lasers is essential. The successful applicant must be willing to work outside normal laboratory hours on a shift basis. Please quote ref. VN 995 in all replies.

There is also a vacancy for a Theoretical Physicist in the Theory and Computation Group of the Laser Division, Rutherford Appleton Laboratory. The group is involved in basic research and the interpretation of experimental data on laser-plasma interactions and laser compression, in laser optical component design interactions and laser compression, in laser optical component design and in the development of computer codes for this work. Most of the group's work is in collaboration with University teams in similar fields. Applicants should hold a good honours degree and have several years experience in at least one of the topics listed above, together with some experience in numerical techniques. Please quote ref. VN 996 in all replies.

The successful applicants will be appointed to the Higher Scientific Officer Scale of £6,530 to £8,589.

Application forms can be obtained from the Recruitment Office, Personnel Group on Abingdon (0235) 21900, Ext. 510. Alternatively, write to her quoting the relevant reference number, to Rutherford Appleton Laboratory, Chilton, Didcot, Oxon. OX11 0QX.

Closing date for applications: 16th December 1981.

(020)A

nthe forefront
of research

# SIMON FRASER UNIVERSITY

Burnaby, British Columbia, Canada

DEPARTMENT OF BIOLOGICAL SCIENCES

#### **ENVIRONMENTAL** TOXICOLOGY

Applications are invited for two tenure-track positions, one at the Assistant Professor level and one at the Associate Professor level. The successful applicants will teach undergraduate courses in Environmental Toxicology and graduate courses in Industrial Toxicology and Food and Drug Toxicology. Candidates must have a PhD degree with experience in toxicology and a strong record in research. The successful applicants will be expected to develop and maintain an active research programme in an area of toxicology appropriate to a Life Sciences department. Preference will be given to candidates eligible for employment in Canada at the time of application.

The positions are available from 1 September 1982 subject to budgetary constraints. The current salary base for the Assistant and Associate Professor ranks are \$25,000 and \$31,600/yr respectively.

Applications should include a curriculum vitae, a brief statement of research interests and objectives, and research interests and objectives, and research. Applicants should request confidential assessments of their research and teaching ability from three referees, to be forwarded directly to: Dr K K Nair, Chairman, Dept of Biological Sciences Dept of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6

Dealine for receipt of applications is 31 January 1982, or positions are filled. (N or until the (NW129)A

# **AGRICULTURAL** RESEARCH COUNCIL

#### SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC **OFFICER**

#### Weed Research Organization

To develop a programme to study the effect on phytotoxicity of the physical form in which a herbicide reaches the plants. An important aspect will be activity following transfer in the vapour phase. A biophysical approach to the problem is required.

Candidates must possess a first or upper second class honours degree in a biological science, chemistry or physics with at least two years relevant post-qualifying experience for appointment to the higher grade. Salary on Civil Service scales.

For further particulars and application form write to the Secretary, Weed Research Organisation, Begbrooke Hill, Yarnton, Oxford OX5 1PF quoting 15/81. Completed applications are to be returned by 24th December 1981.

## STATENS SERUMINSTITUT (Copenhagen)

### **DEPUTY HEAD** OF DEPARTMENT

A position is available in the Vaccine Department from 1st February 1982 or as agreed upon. Responsibilities will include routine functions, and basic and applied research. The Department produces bacterial vaccines, purifies immunoglobulins, completes other products and purchases vaccines and immunoglobulins from abroad.

The Department, which is impliine Department, which is implicated in an advisory capacity in questions relating to vaccines and vaccination and in postgraduate education, is a WHO Collaborating Centre for Research and Reference Services for Immunological Biological Products and WHO Laboratory for Biological Standards. In the future the Department will be In the future the Department will be responsible, in collaboration with the other production departments, for the entire production and control of vaccines and will probably be incorporated with one or several departments in a production section, at a later date.

Candidates should be conversant with production and control of biological products. Experience in microbiological, biochemical and/or immunological methods would be an advantage.

The ideal candidate is a person of roven research ability with a flair for leadership.

The position is permanent, salary 271.500 Danish kroner. The work will be carried out at Statens Seruminstitut, but the appointment is under the Ministry of the Interior.

Applications should be addressed to Her Majesty the Queen and five copies should be sent to the Directors, Statens Seruminstitut, Amager Boulevard 80, DK-2300 Copenhagen S, by 4 pm 8th January 1982

(W523)A

### RESEARCH APPOINTMENT Applications are invited from ORGANIC CHEMISTS OR **BIOCHEMISTS**

to join a group working on the structural aspects of protein-nucleic acid interactions. The project involves the synthesis and examination of model systems by X-ray crystallography and other physical methods. While previous direct experience in this field is not required, candidates should have good experimental ability. Postdoctoral qualifications helpful but not essential.

The post is supported by a long term MRC grant. Initial appointment term MRC grant. Initial appointment for two years with possibility of renewal. Salary dependent on age and qualifications. Please apply in writing, including cv and names of two referees to Dr Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. (9995)A

A CELL Biologist is required in the Molecular Pharmacology Department for a project concerned with chemically induced neoplastic transformation and mutation in mammalian cells in culture. Experience of tissue-culture methodology and other cell biological procedures would be advantageous. Knowledge of German is not necessary. The salary is approximately DM 4,000 BAT IIa). The appointment is to begin as soon as possible. Applications to: Professor Dr Oesch, Department of Pharmacology, University of Mainz, Obere Zahlbacher Strasse 67, 6500 Mainz, West-Germany. (W514)A

#### **MOLECULAR BIOLOGIST/** BIOTECHNOLOGY

NORDISK GENTOFTE, Copenhagen, Denmark is currently expanding its research and development programme in the field of bio-technology. Applications are invited from scientific personnel with interest, qualifications, and experience within

- DNA technology
- cloning of eukaryotic genes
- expression of cloned genes in various hosts

fermentation technology. NORDISK GENTOFTE manufactures and markets insulins, plasma products, human growth hormone etc. and makes up the independent foundation NORDISK INSULINLABORATORIUM together with HAGEDORN RESEARCH LABORATORY (Basic research into diabetes) and STENO MEMORIAL HOSPITAL (research and treatment

of diabetes).
NORDISK GENTOFTE is conveniently situated in the northern part of Copenhagen.

For further information please contact Dr. H. H. Dahl (telephone:

(45) 1 680168 ext 432).

Applications with curriculum vitae should be sent to: NORDISK GENTOFTE, Niels Steensensvej 1, DK-2829 Gentofte, Denmark

(W519)A

#### **GEOPHYSICAL FLUID** DYNAMICIST/PHYSICAL **OCEANOGRAPHER**

Applications are solicited for a junior faculty position in ocean physics or dynamics to begin in the academic year 1982-83. Areas of interest to the Department include analytical, numerical and laboratory modeling of physical processes and phenomena in the sea.

Yale University is an equal opportunity/affirmative action employer and encourages women and members of minority groups to compete for this position.

Curriculum vitae, publications, and the names of three or more referees should be sent by 31 December 1981 to: Robert B Gordon, Chairman, Department of Geology and Geophysics, PO Box 6666, New Haven, CT06511. (NW133)A

### **GENETICS LABORATORY** DEPARTMENT OF BIOCHEMISTRY POST-DOCTORAL

RESEARCH ASSISTANT **GRADE IA** (Ref: G/R/46)

A position is available for two years to work on human complement variants using electrophoretic techniques and serological techniques. Consideration will be given to Post-Graduates with relevant experience. Part time work might be considered for exceptional applicants.

Salary on the scale £6,070 pa -£7,290 pa.

Applications, including a full cv and the names and addresses of two referees, should be sent as soon as possible to: The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU.

(9996)A

#### THE MATHILDA AND TERENCE KENNEDY INSTITUTE OF RHEUMATOLOGY

#### POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a Post-Doctoral Research Assistant in the Division of Biochemistry (Head, Dr Helen Muir, FRS). The successful applicant will be required to develop methods for the production of mono-clonal antibodies to lysosomal enzymes as part of a project involving an investigation into the ability of different types of cell to exchange lysosomal enzymes

Previous experience in immunological and/or cell culture techniques is required. The position is funded for 3 years starting salary up to £7,847 including London Weighting on Range IA of the Research Staff scales of London University.

Applications. including curriculum vitae and the names of two referees, should be addressed to: The General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, 6 Bute Gardens, Hammersmith, London W67DW.

# **UNIVERSITY OF CAPE TOWN ASSOCIATE PROFESSOR OR** SENIOR LECTURER IN GENETICS

Applications are invited for the above post in the Department of Biochemistry. Appointment, according to qualifications and experience, will be made on the salary scale:

Senior Lecturer: R14,370 × 810-R20,850 pa. Associate Professor: R18.420 × 810-R20,850 × 900-R22,650 pa. In addition a service bonus of nearly one month's salary is payable annually.

Candidates should have strong research interest in eucaryotic molecular genetics and experience in recombinant DNA technology and assume duties during the first half of 1982. Candidates should state for which post they wish to be considered.

The post may also be filled at the request of the applicant on a contract basis for 2-3 years with either the possibility of renewal or conversion into a tenured post.

Applicants should submit curriculum vitae, stating present salary, research interest and publications, when available if appointed, and the names and addresses of three referees.

Further information should be obtained from The Registrar (Attention Appointments Officer) University of Cape Town, Rondebosch, 7700 South Africa to whom applications (Quoting reference No AC/11) must be submitted as soon as possible.

The University's policy is not to discriminate in the appointment of staff on the grounds of sex, race or religion. Further information ont he implementation of this policy is obtainable from the Registrar. (W518) A

#### THE HANNAH RESEARCH INSTITUTE

(for studies relating to the production and utilization of milk) CHEMIST

Applications are invited for a vacant post in the Chemistry Department to undertake studies on the physical properties of milk proteins absorbed onto fat surfaces and the properties of homogenized protein/fat systems. Applicants should possess a first or upper second class honours degree in Chemistry, Biophysics of a related subject and should have at least two

years post-qualifying experience. Appointment would be to the Higher Scientific Officer grade salary scale £8,589. A non-contributory superannuation scheme is

operating.

Further particulars may be obtained from: The Secretary, The Hannah Research Institute, AYR KA6 5HL. to whom applications, including curriculum vitae and the names and addresses of two referees, should be forwarded by 4th January, 1982 quoting reference HRI No. 49.

(015)A

M.R.C. CLINICAL RESEARCH CENTRE (NORTHWICK PARK HOSPITAL) WATFORD ROAD, HARROW, MIDDLESEX HA13UJ

# Electron Microscopist — Cytochemist

We have a vacancy for a non-clinical scientific research worker to work jointly between the division of clinical cell biology and the histopathology section. The work involves developing and applying enzymic methods at an ultrastructural level and the use of techniques in the study of human pathology. The successful candidate would be expected to develop new em cytochemical techniques applying biochemical assays for the localisation of specific tissue phosphatase to both subcellular fractions and whole tissues.

The appointment is for a maximum of 3 years subject to confirmation after the first year and is intended for candidates of immediately post-doctoral or equivalent status, or more senior individuals with less than 3 years post-doctoral experience. Salary within the range £8,296  $-\,$  £13,827 per annum (inclusive of London Weighting) depending on age and experience.

For further information contact Dr T. Peters on 01-864 5311 ext 2139. Apply in writing to Miss B. Shaw for application forms quoting ref 104/1/3963. Closing date for applications 31st December 1981.

# STATENS SERUMINSTITUT (Copenhagen)

# HEAD OF DEPARTMENT

position is available for a professional and administrative head of the Vaccine Department from 1st February 1982 or as agreed upon. Responsibilities will include routine functions and basic and applied research.

The Department produces bacterial vaccines, purifies immunoglobulins, completes other products and purchases vaccines and immunoglobulins from abroad.

The Department, which is implicated in an advisory capacity in questions relating to vaccines and vaccination and in postgraduate education, is a WHO Collaborating Centre for Research and Reference Services for Immunological Biological Products and WHO Laboratory for Biological Standards. In the future the Department will be responsible, in collaboration with the other production departments, for the entire production and control of vaccines and will probably be incorporated with one or several departments in a production section, at a later date.

Candidates should be conversant with production and control of biological products. Experience in microbiological, biochemical and/or immunological methods would be an advantage.

The ideal candidate is a person of proven research ability with a flair for leadership.

The position is permanent, salary 291.700 Danish kroner. The work will be carried out at Statens Seruminstitut, but the appointment is under the Ministry of the Interior.

Applications should be addressed to Her Majesty the Queen and five copies should be sent to the Directors, Statens Seruminstitut, Amager Boulevard 80, DK-2300 Copenhagen S, by 4pm 8th January 198

(W521)A

# **STUDENTSHIPS**

# **TECHNICIANS**

### **BIOCLONE LIMITED** West Yorkshire

scientists and technicians

Applications to join this new venture and develop the exciting opportunities offered by this field are invited from persons experienced in hybridoma generation; tissue culture; immunoassays or protein

Further details from Dr J P Dickinson on (0532) 788042 or from Bioclone Ltd, 3, Hockney Road, Bradford BD8 9HQ, West Yorkshire, to whom applications (CV and 3 referees) should be addressed. (009)A

a curriculum vitae, a summary of proposed research, and the names of Carothers, Search Committee Chairman, Department of Botany, University of Illinois, 505 South Urbana, Illinois Urbana, 61801-3793; telephone (217) 333-3261. In order to ensure full consideration, applications must be received by 1 February 1982. The University of Illinois is an Affirmative Action/

# **SCIENTISTS AND**

This new company, dedicated to expansion of the production and use of monoclonal antibodies, will shortly be appointing several

PLANT Molecular Biology. The Department of Botany, University of Illinois at Urbana-Champaign, invites applications for a tenure-track position at the assistant professor level for appointment in August 1982, subject to budgetary approval. Applicants must have a PhD in plant biology/biochemistry and research interest in genetic engineering as it relates to the basic biology of gene expression in plants and/or the modification of plant genomes. The appointee will be expected to do research in the above area and to contribute to the graduate and undergraduate teaching programs. Salary is open, depending on the applicant's qualifications. Applicants should send at least three references to: Dr Zane B Equal Employment Opportunity Employer. (NW121)A

# SEMINARS and SYMPOSIA

SEMINARS and SYMPOSIA

35th Annual Symposium on Fundamental Cancer Research

"Perspectives on Genes and the Molecular Biology of Cancer"

March 2-5, 1982

This symnposium, sponsored by The University of Texas M D Anderson Hospital and Tumor Institute, will focus on the molecular biology of cancer and explore in depth the metabolism involved in cellular cancer growth. Over 30 lectures and several poster sessions will cover the following topics:

• Gene organization and evolution
• Regulation of gene expression
• Gene transfer
• Novel applications of recombinant DNA technology to human cancer

Speakers will include:
R. Axel, D. Baltimore, J. Baxter, P. Berg, M. Birnstiel, D. Botstein, D. Brown, P. Chambon, D. Clayton, R. Davis, B. DeCrombrugghe, W. Gilbert, W. Hazeltine, L. Hood, R. Kamen, P. Leder, T. Maniatis, B. Mintz, B. O'Malley, D. Robberson, F. Ruddle, G. Saunders, R. Scimke, C. Schmid, P. Sharp, H. Temin, S. Tonegawa, G. Vande Woude, H. Varmus, R. Weinberg, C. Weissman, and R. White.

For scientists interested in presenting poster papers, please send abstracts by Dec. 31, 1981 to

Dr Grady F. Saunders or Dr Donald L. Robberson M.D. Anderson Hospital and Tumor Institute 6723 Bertner Ave.
Houston, Tex. 77030

Registration information may be obtained from Frances Goff, Room 115, at the same address.

(NW132)M

### **CONFERENCES** and COURSES

# **ROYAL SOCIETY OF CHEMISTRY Residential Schools**

"Inductively Coupled Plasma Emission Spectrometry". 22-24 March 1982, U.M.I.S.T.

"Enzymology — Applications and Modern Practice", 19-23 April 1982, University of Exeter.

"High Performance Liquid Chromatography", 5-9 July 1982, University of Sussex.

> "Heterocyclic Chemistry", 6-8 September 1982, University of Sussex.

"Pollution: Causes, Effects and Control". 13-15 September 1982, University of Lancaster.

Further details and application forms from: Miss L. Hart, Royal Society of Chemistry, 30 Russell Square, London WC1B 5DT. Tel: 01-631 1355. (9984)C



KELSTERTON COLLEGE, CONNAH'S QUAY, CLWYD

**RESEARCH DIVISION** 

Applications are invited for a

# RESEARCH STUDENTSHIP

tenable for three years at SRC equivalent grant levels. The Research Project will involve free radical chemistry and photochemistry using the fast reaction techniques of laser flash photolysis and pulse radiolysis. Applicants should have a good honours degree (or equivalent) in Chemistry and Biochemistry.

Further details and application form available from the Institute Registrar, Kelsterton College, Connah's Quay, Clwyd. Tel: Deeside 817531 ext 271.

Closing date for receipt of applications within 14 days of the appearance of this advertisement.

## **COLOUR VISION**

A NATO meeting concentrating on the psychophysics and electro-physics and electrophysiology of human and primate colour vision will be held at Cambridge University, August 23-27, 1982, For details please write to Dr. J. D. Mollon, Department of Experimental Psychology, Downing Street, Cambridge CB2 3EB, United Kingdom. (9972)C

# UNIVERSITY **OF NOTTINGHAM** MSc COURSE IN MEAT SCIENCE

Applications are invited from Graduates in appropriate subjects (normally in Agricultural, Bio-logical, Chemical or Veterinary Sciences) for admission to an advanced course of study leading to the degree of MSc (by examination) in Meat Science. The course, which is of one year's duration, includes Production of Meat Animals, Physiology of Meat Animals, Animal Health & Meat Inspection, Commodity Science of Meat and the Economics and Marketing of Meat. A short dissertation based on research of a topic selected from one of these 5 subjects constitutes part of the overall assessment for the degree.

The course is designed to provide those having appropriate scientific training with specialist knowledge of meat whereby they would be suitably qualified to accept responsibility for the scientific control of meat as a commodity in relation to developments within EEC and in world trade generally.

For suitable UK applicants, scholarships may be available.

Those seeking admission for the 1982-83 session should write before February 1st 1982 requesting an application form, from the following:

The Secretary (Ref PG P), School of Agriculture, University of Nottingham, Sutton Bonington, Loughborough, Leics LE12 5RD (9992)C

# **STUDENTSHIP**

**QUEEN ELIZABETH** COLLEGE (University of London) **DEPARTMENT OF PHYSIOLOGY** 

# MICROELECTRONICS EDUCATION PROGRAMME RESEARCH STUDENTSHIP

Applications are invited from graduates with at least second class honours who can combine a biological science training with knowledge of strong interests in the use of microcomputers for a two year studentship investigating the use of microcomputers in physiological and other biological science for classroom experiments. The successful candidate will be required to register for a research degree of University of London.

Curriculum vitae should be sent as soon as possible to Dr I C H Smith at the Department of Physiology, Queen Elizabeth College, Campden Hill Road, Kensington, London W8 (9980)F

# **FELLOWSHIPS**

# 1982 BEIT MEMORIAL **FELLOWSHIPS FOR** MEDICAL RESEARCH

Notice is hereby given that an election of Junior Beit Fellows to begin work on 1 October 1982 will take place in May 1982. Junior Fellowships carry an initial value of £6,880 - £8,515 plus £967 London Allowance, plus yearly increments for three years. There will be the usual university superannuation contributions and benefits. Candidates must have taken a degree in a faculty of a university approved by the Trustees in Her Majesty's Dominions, Protectorates and Mandated Teritories, India, Pakistan, the Republic of Ireland and the United Kingdom, or a medical diploma registrable in the UK. Elections to Junior Fellowships are rarely made above the age of 35 years.

Applications from candidates must be received not later than 19 March 1982. Candidates must submit evidence where they propose to work, which must be in Great Britain or

Forms of applications and all information may be obtained from Professor W G Spector (Secretary), Pathology Department, St Bartholomew's Hospital, London EC1A 7BE. For overseas candidates forms of application may be obtained from, The Secretary, South African Medical Council, PO Box 205, Pretoria, South Africa; The Ministry of Health, The Government of India, New Delhi, India; The Secretary, Department of Education and Science, PO Box 826, Canberra, City, ACT 2601, Australia; The Department of Health, Wellington, New Zealand; and, The Canadian Medical Association, 1867, Alta Vista Drive, Ottawa 8, Ontario, Canada.

# IMPERIAL CANCER **RESEARCH FUND** RESEARCH FELLOWSHIP

Applications are invited for a three year postdoctoral appointment to work with Dr Ian Kerr on the biochemistry of interferon action, the regulation of the interferon response and the role of the 2-5A system in relation to its wider possible significance in the control of cell growth and development. Experience with proteins and nucleic acids or genetic engineering techniques or membrane biochemistry and some familiarity with handling cells in tissue culture are desirable.

The position is available immediately and candidates should be free to take up the appointment by September 1982 at the latest. Salary range £8,787 to £10,837 (inclusive of London Allowance), with entry according to qualifications and experience.

For further information contact Dr Ian Kerr (tel. 01-242 0200 ext 337). Applications with full CV and the names and addresses of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 by 1st Junuary 1982 quoting ref. 8/82. (9931)F

# **EMBO**

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

# SHORT TERM FELLOWSHIPS in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of the application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatiantic travel are awarded only in extremely exceptional circumstances

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G.

# Vincent C. Kelley and Leon T. Silver **GRADUATE FELLOWSHIPS** DEPARTMENT OF GEOLOGY THE UNIVERSITY OF NEW MEXICO

The Department of Geology of the University of New Mexico invites applications for the Vincent C. Kelley and Leon T. Silver Graduate Fellowships. The fellowships will be awarded on the basis of the scholastic record and academic promise of graduate applicants. Each fellowship will provide for a generous living stipend of \$1,000/ month for 9 to 12 months, and up to \$2,000/year for travel and research expenses. The Caswell Silver Foundation will pay all tuition and university fees. The awards are made on an annual basis, but may be renewed for up to three years as long as the student maintains excellent academic standing and shows evidence of significant progress in research. Preference will be given to, but is not restricted to, applicants for the PhD program An application for admission to the UNM Graduate Program, tran-Graduate Record Exam scripts, results (verbal, math & geology), three letters of reference and a brief statement of research goals are required for consideration for the fellowships. Application materials may be obtained from: Rodney C. Ewing

Chairman Department of Geology University of New Mexico

Albuquerque New Mexico 87131

The deadline for applications is March 1, 1982 for the Fall semester (NW131)E of 1982.

Please mention

# nature

when replying to these advertisements

# IMPERIAL CANCER RESEARCH FUND RESEARCH FELLOWSHIP

Applications are invited for a three year postdoctoral appointment to clone mammalian DNA sequences specifying control regions, structural genes and transforming genes, and to test the expression and biological activity of the cloned sequences by the use of DNA transfection techniques and eukaryotic vector systems.

Salary range £8,787 to £10,837 (inclusive of London Allowance) with entry according to qualifications and experience.

For further information contact Dr M Fried (Tel. 01-242 0200 ext 297). Applications with curriculum vitae and the names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 England quoting reference no. 9/82. (9932)E.

# **POSTDOCTORAL FELLOWSHIPS IN IMMUNOLOGY**

Position available postdoctoral training in basic immunology (idiotypic regulatory mechanisms) and applied problems (tumor immunology and leukemia virislymphocyte interactions). Other related activities in the department include research on biology of interleukins, interferons, and cellular immunity (cytotoxic and NK cells).

Send complete CV and references to: Jan Cerny, MD, PhD, Department of Microbiology University of Texas Medical Branch, Galveston, Texas, 77550.

An Equal Opportunity/Affirmative Action Employer.

(NW137)F

# FIRST ANNOUNCEMENT EIGHTH EMBO ANNUAL SYMPOSIUM 1982 GENETIC FLUX

# 3-7 October 1982 EMBL Heidelberg (F.R.G.)

The seven plenary sessions will cover: Transposition in prokaryotes and eukaryotes; the integration of viruses and DNA; switching gene functions; gene families; repeated sequences; the structure of organelle genomes; and the evolutionary implications of genetic flux.

In addition to members of the Organizing Committee the speakers will include: D. Baltimore (Cambridge-USA); C. Blake (Oxford-UK); B. Burr (Upton-USA); J. Carbon (Santa Barbara-USA); I. Dawid (Bethesda-USA); G. Felsenfeld (Bethesda-USA); G. R. Fink (Ithaca-USA); M. Fried (London-UK); W. J. Gehring (Basel-Switzerland); W. Gilbert (Cambridge-USA); R. Jaenisch (Hamburg-FRG); A. Jeffreys (Leicester-UK); R. Kahmann (Martinsried-FRG); J. Maynard Smith (Brighton-UK); H. A. Nash (Bethesda-USA); K. A. Nasmyth (Cold Spring Harbor-USA); P. Philippsen (Basel-Switzerland); K. Rajewsky (Köln-FRG); H. Saedler (Köln-FRG); J. Schell (Köln-FRG); M. Singer (Bethesda-USA); F. Stahl (Eugene-USA); G. Stark (Stanford-USA); R. E. Streek (München-FRG); S. Tonegawa (Cambridge-USA); A. Toussaint (Brussels-Belgium); R. A. Weinberg (Cambridge-USA); K. Willecke (Essen-FRG).

The Symposium will be held at the European Molecular Biology Laboratory, Heidelberg, with registration on Sunday, 3 October 1982. Plenary sessions will be held in the mornings and late afternoons of 4-6 October and the morning of 7 October. There will be three poster sessions in the afternoons of 4-6 October inclusive. Participants will be accommodated in hotels in Heidelberg, and a small number of inexpensive rooms will be available in the EMBL guesthouse. The registration fee (see below) does NOT cover the cost of accommodation.

Applications for participation, including a curriculum vitae and a brief description of current research interests should be sent to: Dr. J. Tooze, Executive Secretary, EMBO, Postfach 1022.40, 69 Heidelberg, F.R.G. The registration fee is DM 180, for graduate students DM 90, and for participants from industry DM 360. Applications should reach Heidelberg by 15 June 1982. Since the number of participants is limited to 220, the Organizing Committee will notify those who have been accepted in August.

Organizing Committee: W. Arber (Basel-Switzerland), G. Bernardi (Paris-France), W. Bodmer (London-UK), P. Borst (Amsterdam-Netherlands), P. Starlinger (Köln-FRG), and J. Tooze (Heidelberg-FRG). (W513)E

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Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is February 19, 1982. Successful candidates will be notified of the their awards immediately after the meeting of the selection committee which is on April 30, 1982.

Application forms and further details may be obtained from Dr J Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1,F.R. Germany.

(W510)E

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Applications should reach the College Secretary (from whom further details may be obtained) between 1st and 31st January, and should include a curriculum vitae with a bibliography and the typescript of any unpublished work, an explanation of the nature of the proposed research, and the names of two referees. Candidates will be expected to make themselves available for interview in the last week of February. The College will bear the cost of travel within the British Isles. (9971)E

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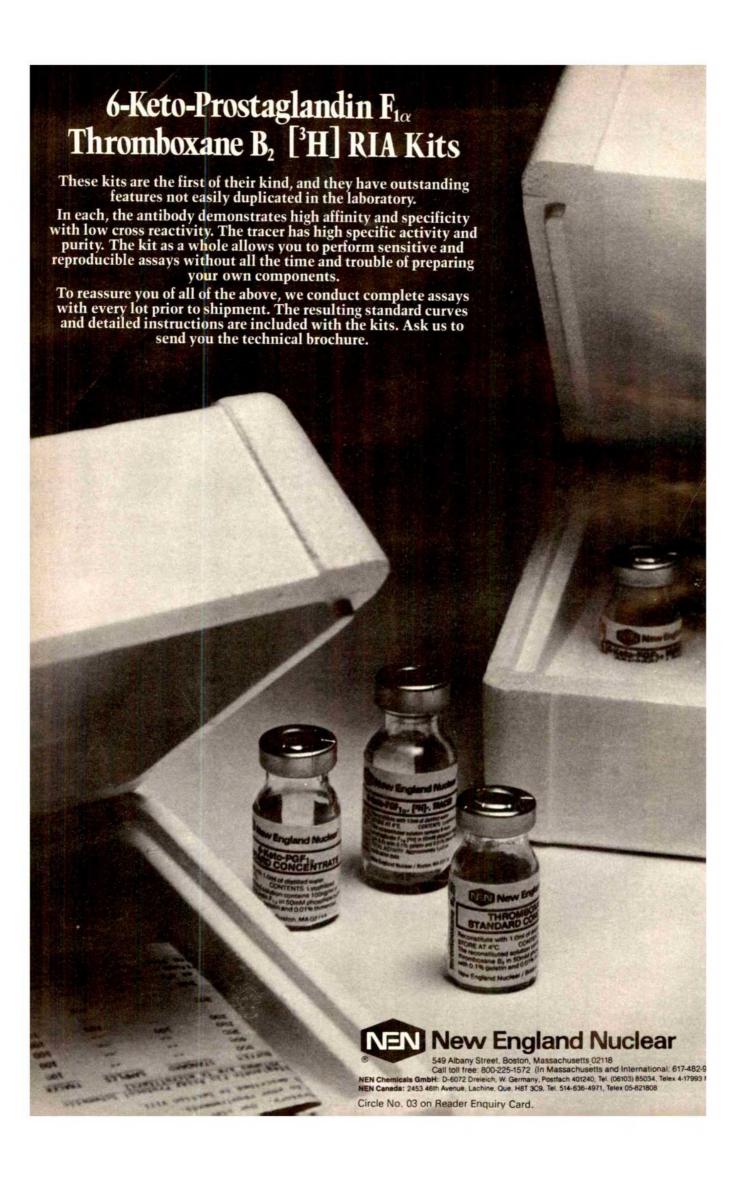
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Further particulars from Assistant Registrar, (Sci & Eng), PO Box 363 Birmingham B15 2TT to whom applications (3 copies) including curriculum vitae and naming three referees should be sent by *Thursday*, 31 December, 1981.

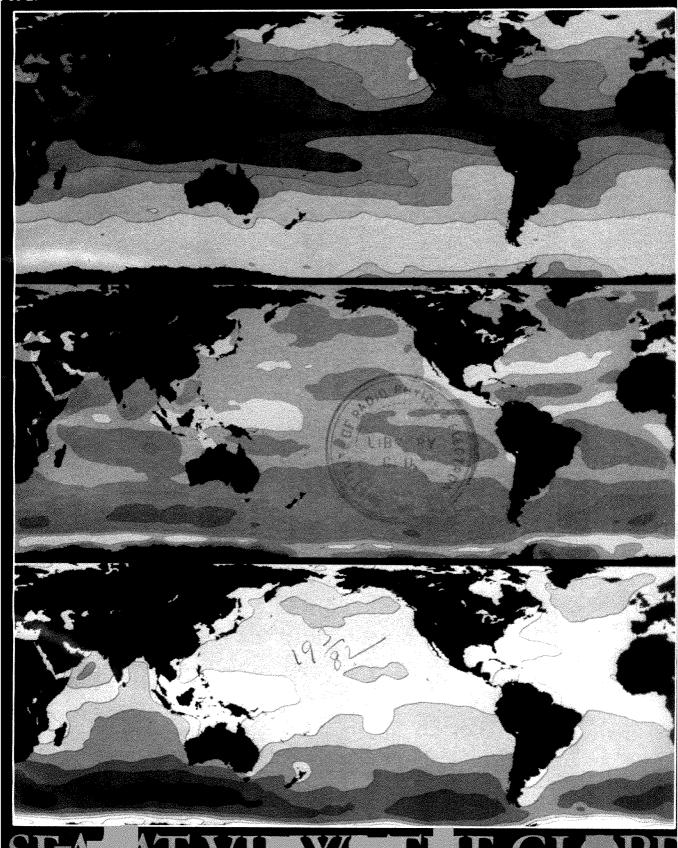
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TATE OF THE SECOND SECO			
EMBO COURSES 1982			Date
Provisional Programme	Overenizer and	Address for Further Information and Inquiries	Place Number of
Subject PRACTICAL COURSES	Organizer and	Address for rurmer information and inquiries	Participants
Microinjection	Professor	Institut für Molekularbiologie	15-19 March
and the second of the second	A. Gräßmann	und Biochemie (WE 03) Freie Universität FB1, WE3	Berlin 12-15
		Arnimallee 22 1 Berlin 33	
		FEDERAL REPUBLIC OF GERMANY	0.470
Gene Cloning, Expression and Mutagenesis	Dr. M. Zabeau	European Molecular Biology Laboratory Postfach 1022.09	6-17 September Heidelberg
and mulagerious		69 Heidelberg FEDERAL REPUBLIC OF GERMANY	20
Novel Developments in Rapid DNA	Dr. G. Volckaert	Rega Institute for Medical Research	10 days
Sequencing and Synthesis Techniques		University of Leuven Minderbroedersstraat 10	in September Leuven
isciniques		3000 Leuven	20-25
Chromosome Dissection, DNA	Dr. JE. Edström	BELGIUM European Molecular Biology Laboratory	6-17 June
Microcloning and Microinjection		Postfach 1022.09 69 Heidelberg FRG	Heidelberg 6-8
Molecular and Cellular Biology	Dr. F.R. Opperdoes	Research Unit for Tropical Diseases	13-25 Septembe Brussels
of Trypanosomes		ICP Av. Hippocrate, 74	20
		1200 Brussels BELGIUM	
Plant Cell Culture Techniques	Dr. I. Potrykus	Friedrich Miescher Institut	Autumn
for Molecular Biologists		Postfach 273 4002 Basel	Basel 15
	Danifer ::-	SWITZERLAND	24-28 May
Immunocytochemistry and its Application in Brain Research	Professor D.F. Swaab	Netherlands Institute for Brain Research	Amsterdam
• •		ljdijk 28 1095 KJ Amsterdam	15
ou Nilve		NETHERLANDS	
Hybridomas and Monoclonal Antibodies	Dr. Z. Eshhar	Department of Chemical Immunology Weizmann Institute of Science	27 June-13 July Rehovot
Antibodies		Rehovot 76100	15
Chromosomal Localization	Dr. G. Bernardi	ISRAEL Laboratoire de Génétique Moléculaire	3rd May-11th M
of Genes	Dr. G. Domera	IRBM	Paris
		2, place Jussieu 75005 Paris	50
e Maria		FRANCE	04 44
Automated Chemical and Enzymatic Gene Synthesis	Professor H.G. Gassen	Institut für Organische Chemie und Biochemie	21 March-3 April Darmstadt
		Technische Hochschule Petersenstrasse 22	15
		61 Darmstadt	
Computer Image Processing of	Dr. K.R. Leonard	FEDERAL REPUBLIC OF GERMANY European Molecular Biology Laboratory	21-28 April
Electron Micrographs	Dr. 16,11, Loonard	Postfach 1022 09	Heidelberg
		69 Heidelberg FEDERAL REPUBLIC OF GERMANY	16-20
DNA Nucleotide Sequencing Techniques	Dr. F. Galibert	Laboratoire d'Hématologie Expérimentale	3-15 May Paris
techniques		Centre Hayem — Hôpital Saint-Louis 2, place du Dr. Fournier	20
		75475 Paris Cédex 10 FRANCE	
Cloning of Plant Genes	Professor	Department of Physiology	4-16 October
	D. von Wettstein	Carlsberg Laboratory Gamle Carlsberg Vej 10	Copenhagen-Val
	•	250 Copenhagen-Valby DENMARK	
B Lymphocyte Differentiation	Dr. F. Melchers	Basel Institute für Immunologie	4-15 October
		Grenzacherstrasse 487 4056 Basel	8asel 14-16
		SWITZERLAND	
Electron Microscopy of Nucleic Acids	Dr. H. Delius	European Molecular Biology Laboratory Postfach 1022.09	17-22 May Heidelberg
		69 Heidelberg	10
Culture of Neural Cells	Professor	FEDERAL REPUBLIC OF GERMANY Department of Anatomy & Embryology	June/July
	G. Burnstock	Centre for Neuroscience	London
		Gower Street London WC1E 6BT	15
The Use of Ti Plasmid as	Dr. M. Van	ENGLAND Laboratorium voor Genetika	9-27 August
Cloning Vector for Genetic	Montagu	Rijksuniversiteit	Gent
Engineering in Plants		K.L. Ledeganckstraat 35 9000 Gent	16
		BELGIUM	
LECTURE COURSES	D- 0 H	0	
Drosophila Developmental Genetics	Dr. G. Morata	Centro de Biologia Molecular Facultad de Ciencias	30 June-7 July Madrid
		Canto Blanco	50
<u> </u>		Madrid-34 SPAIN	
Regulation of Gene Expression in Prokaryotes and Eukaryotes	Dr. M. Grunberg- Manago	Institut de Biologie Physico- Chimique	30 August- 11 September
(cosponsors NATO and FEBS)	man mgr	13, rue P. et M. Curie	Spetsai
• •		75005 Paris FRANCE	110
Single Channel Recording in Biological Membranes	Professor F. Conti	Laboratorio di Cibernetica &	20-29 May
्र		Biofisica, CNR Camogli	Erice, Trapani 12
Intensive Lecture Course:	Dr. A. Siccardi	ITALY Istituto di Genetica	
Basic Immunology	M. C. SRUGIUI	via S. Epifanio, 14	13-25 September Pavia
		27100 Pavia ITALY	60



# nature

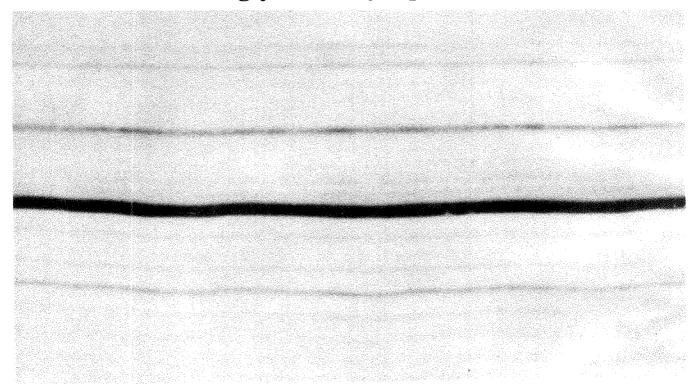
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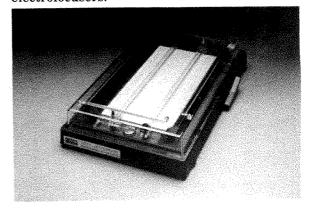
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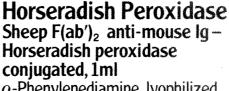
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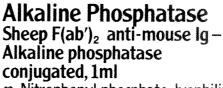




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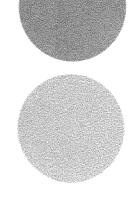
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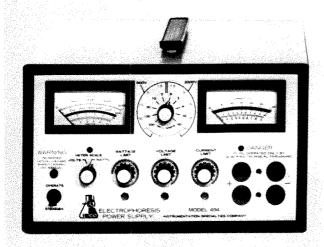
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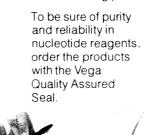
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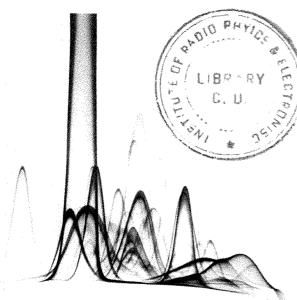
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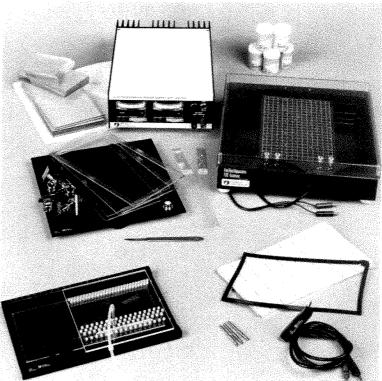
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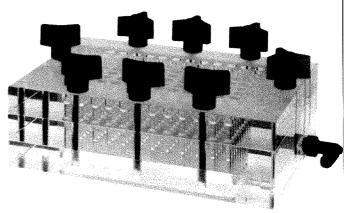


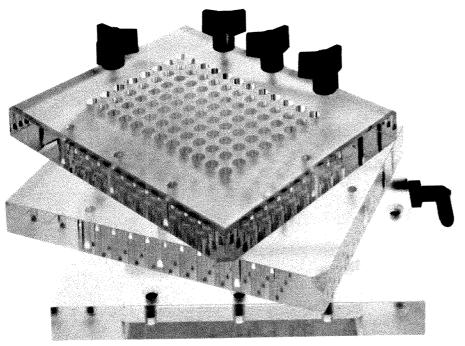
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Not a revolution, more a Trojan horse 503

# NEWS

US dispute on nuclear bomb safeguards Is creation a science or a religion? Germany's breeder Israeli education US research patents Science in France What price IIASA? Pakistan's space ambitions India's space programme European environment Electronic intrigue Animal welfare

Scientific fraud Primitive life in France

# CORRESPONDENCE

Clinical psychology/Evolution etc.

French reorganization

# **NEWS AND VIEWS**

Has corticotropin-releasing factor finally been found? (G Fink) Protein-lipid interactions (A Watts) New low temperature phases of H<sub>2</sub> and D, (J C Raich & R D Etters) New pathways for chlorinated dioxins (A Hay)

Parasites affect behaviour of mice (FEG Cox)

Useful tropical legumes (R M May) Small minded — interplanetary dust and electron microscopy (C Pillinger) Clues for the origin of cosmic rays (P Meyer)

Middle atmosphere dynamics (J Gregory)

# **BOOK REVIEWS**

Otto Warburg: Cell Physiologist, Biochemist and Eccentric (by H Krebs) Hans Kornberg; The Creation (by P W Atkins) John D Barrow; The Nature of Matter (J H Mulvey, ed.) Wolfgang K H Panofsky; The Mammals of North America, 2nd Edn (by E R Hall) Malcolm C McKenna; The Biology of the Coleoptera (by R A Crowson) M E G Evans; Direct Perception (by C F Michaels and C Carello) Oliver J Braddick; Life History of a Fossil (by P Shipman) Mark Newcomer

Averages of atmospheric water vapour (upper panel) wind speed (middle panel) and wave height (lower panel) derived from Seasat during the period 7 July to 10 October 1978. The six water vapour colours are separated by the 1, 2, 3, 4 and 5 g cm<sup>-2</sup> contours, the five wind speed colours by the 5.0, 6.8, 8.6 and 10.4 ms<sup>-1</sup> contours and the seven wave height colours by the 1.3, 2.1, 2.9, 3.7, 4.5 and 5.3 m contours. See page 531 for details. (Pseudo-colour images from the Image Processing Laboratory, Jet Propulsion Laboratory, California Institute of Technology.)

The early Universe (a review)

E W Kolb & M S Turner

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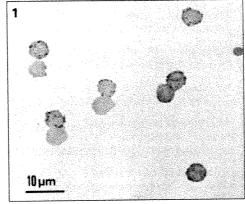
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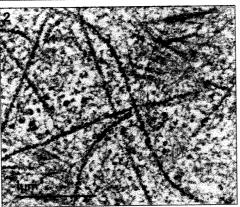
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### References

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- 2. Geoghegan, W.D., et al.: Immunol. Commun. 7, 1 (1977)
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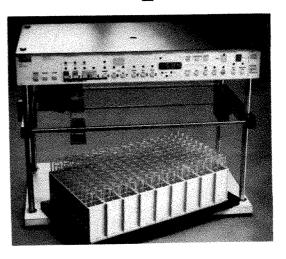
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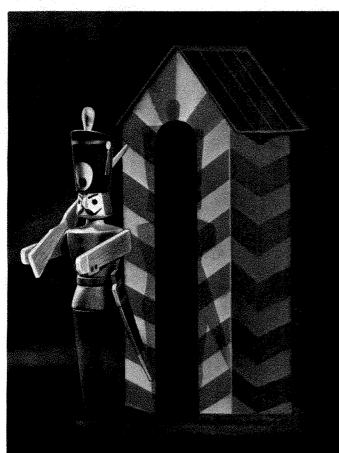






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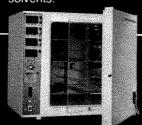
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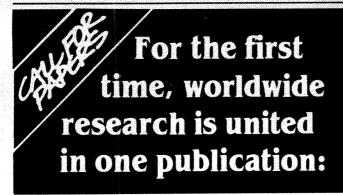
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# nature

# 10 December 1981

# Not a revolution, more a Trojan horse

The House of Lords committee on science and technology has produced a guileful recipe for change. Will the British government listen to what it has to say?

In the nineteenth century, when the British government felt threatened from abroad, the natural response was to build a few more battleships, Now, when most threats are economic rather than strategic, the temptation is to carry through yet another reorganization of the administration of science and technology. Untrue to form, the present British government has been restrained almost to the point of seeming indifferent on the issue, and masterful inaction may indeed be appropriate. For although there is a long-term connection between national prosperity and the efficiency with which research and development is administered, the short-term consequences of tinkering are usually disadvantageous. Yet it is also plain that the machinery of research administration in Britain has for years been out of date. The British government should thus be grateful for the thoroughly sensible report of the House of Lords Committee on Science and Technology, published this Monday. Whether gratitude will flow is naturally quite a different matter. Indeed, from the point of view of a government so embattled on other fronts that it may be forgiven for seeking respite where it can find it, the advice it has received from the House of Lords has the unwelcome attributes of being at once persuasive and practicable.

More clearly than its predecessors, the House of Lords committee has distinguished between the two functions of science (and scientists) in government — the management of research and development and the provision of scientific advice on broader questions of policy. The distinction has been made before, most recently by Dr Martin Holdgate's inquiry into the role of the scientific civil service within the British government, but it has not sunk in. The point is, however, both simple and crucial. Government departments sustain (or should sustain) substantial programmes of research and development and should manage them efficiently. They also need some mechanism for being told from within which policies make scientific sense and which do not. Lorth Rothschild's proposals in 1972 for the reorganization of civil science supposed that if the spending departments of government were provided with chief scientists of sufficient stature, both functions could be carried out by the same people. The experience of the past decade has, however, shown that most chief scientists have been imprisoned by the management of research and development. The House of Lords suggests that this danger could be avoided by appointing two people within each ministry, one to advise on general policy and one to manage the research programme. That is one solution to an immediate problem. A better one would be to ensure that chief scientists should be strengthened, and equipped, to carry out both functions. Either way, something must be done, both departmentally and for central government.

The way in which chief scientists in British government departments have been converted by the pressure of work from putatively subversive agents of change into high-grade clerks is not, however, the only failure of the post-Rothschild era. Even at the outset, it was clear that the Rothschild reorganization entailed the risk of emasculating crucial parts of the previous machinery for providing the government with scientific advice. Thus the old Council for Scientific Policy, which had become fond of writing

(and, to its credit, publishing) reports on matters of general importance, was replaced by the Advisory Board on the Research Councils, a referee to arbitrate between the research councils which appears even to have given up the practice of saying how it purports to do so. The nearest entity within the British government to a central source of advice on science policy is the committee know as the Advisory Council on Applied Research and Development — a busy committee conspicuous chiefly for its lack of power and coherence. The House of Lords committee proposes that this committee should be transformed by some kind of evolutionary process into a Council on Science and Technology, that it should report to some member of the British Cabinet who, while not being a Minister of Science and Technology, would be spokesman in this cause and that the committee should be serviced by the member of the Cabinet Office with responsibilities in this field while its chairman should have access to the nominated minister.

The proposal is sensible. In British terms, it is also devilishly clever. Strengthening a committee is, after all, a good thing to do; setting up a new committee would be unpalatable. Giving an existing minister wider responsibility will seem productive; appointing another would be counted as an encouragement of the growth of bureaucracy. The House of Lords committee has also been politic in the modesty of its requirements of defence research and development. It asks not that there should be a means by which the true ivory tower of British science (defence research) should be coordinated with the rest, but merely that there should be cross-representation on the appropriate committees and that everybody should bend his energies to ensure that what the government spends on defence research should not continue to have no commensurate civil benefit. Cleverly, however, the committee asks that its new council should have some right to ask questions about the interface between defence research and civil industry. It would, of course, have been impolitic to recommend that the committee should have the right to ask what defence research is for

With a little luck — and if the committee's friends do not too eagerly disclose their reasons for believing that it is right — the outcome may be a more intelligent way of running that part of British science and technology supported by the public purse. That is as it should be. Pinstriped gentlemen have for too long been turning up at pointless committee meetings in order to agree that it would be better if there were time to talk about matters of substance, not of procedure or of who gets how much. That the committee's recipe for the administration of civil science is to return to the 1950s, when there was a cabinet minister with parttime responsibility for science and technology and an advisory council whose power derived from its reputation for plain speaking (but not good judgement) is not in itself a reason why the recipe should not be tried. For something, even anything, must be done. It cannot be in the private interests of even British governments that they should feel free to decide, without internal dissent, to rob the academic research base of essential support, discovering only afterwards that they have erred. That, however, is what is now in prospect.

# US dispute on nuclear bomb safeguards

# Agency and Administration at loggerheads

Washington

The State Department and the Nuclear Regulatory Commission (NRC) are drifting towards loggerheads on antiproliferation safeguards. Both parties agree that the safeguards administered by the Vienna-based International Atomic Energy Agency (IAEA) need to be improved. The State Department, reflecting a general drift away from the policies of the Carter Administration, would prefer to use conventional diplomatic channels.

In contrast NRC, which under the Nuclear Non-Proliferation Act signed by President Carter in 1978 is required to assure Congress about the adequacy of IAEA safeguards, is pushing for a more direct approach. Following sharp criticism of IAEA procedures by two former inspectors (Nature 26 November, p.300), the five members of the commission have sent a letter to all members of Congress saying that the current safeguards system "would not detect a diversion in at least some types of facilities".

In the light of this concern and the general shadows that have recently been cast over the adequacy of IAEA safeguards, NRC has decided to carry out a review of its own policies on granting export licences for nuclear materials. The State Department, however, although embarrassed by disclosures about weaknesses in IAEA's safeguards system, does not seem to feel any such drastic action is necessary. Addressing the Senate Foreign Relations Committee last week, Mr Richard T. Kennedy, Under-Secretary of State for Management and a former member of NRC, repeated the Administration's commitment strongly to support the agency.

Introducing the Senate hearings, committee chairman Senator Charles Percy emphasized the central importance of IAEA, and pushed Mr Kennedy hard on whether the Administration was doing everything it could to prevent the spread of nuclear military technology, complaining that the Senate had been provided with insufficient information on procedures agreed with Pakistan and IAEA to prevent the diversion of civilian technology. Senator Percy put the suggestion strongly denied by Mr Kennedy — that the Administration might be going "soft" on non-proliferation to help boost the sagging exports of the domestic nuclear industry.

Democrat Senator John Glenn went

even further, suggesting that the State Department should put pressure on other industrialized countries to provide a significant increase in technical and financial resources for the agency. To support his argument that such increases were badly needed, Mr Glenn produced a report prepared at the Pacific Northwest Laboratory under contract to the Department of Energy throwing doubt on whether IAEA was able to achieve its technical objectives of the surveillance and accounting of nuclear materials.

One issue on which there was little disagreement between Senator Percy and the Administration was over attempts by Third World countries to gain greater influence on the activities of IAEA, both in terms of increasing their membership of the governing council and in appointing more inspectors from the developing nations. On the latter point it was agreed that, although an important objective, it should not be pursued at the price of sacrificing the quality of inspection procedures. On the other hand, Mr Kennedy made it clear that the State Department would resist attempts to "politicize" the agency, in terms of changing the current balance of control.

There is a deeper division over the extent to which a lack of detailed information about the technical data used by IAEA in

# Is creation a science or a religion?

Little Rock

The state of Arkansas was accused on Monday morning of making "an unprecedented attempt to legislate what is science" at the opening of a trial over its efforts to determine how different theories of the origins of man should be taught in its public schools.

The American Civil Liberties Union (ACLU) is challenging as unconstitutional a law, passed by the state legislature earlier this year, which requires that whenever the Darwinian theory of evolution is taught, an equal amount of time should be devoted to presenting evidence in favour of what is described as "creation science".

ACLU contends that the law violates the first amendment to the constitution, which requires a strict separation of church and state. It argues, in the words of Little Rock attorney Robert M. Cearley, that "creation science is not science but religious apologetics, an attempt to prove or justify sectarian religious beliefs". The state argues that there is nothing inherently religious in teaching that the world came into being as the result of a positive act of creation, and that the evidence used to support this hypothesis is "at least as scientific" as the evidence for the "evolution science".

Setting the agenda for a debate that is likely to dominate the trial, state attorneygeneral Stephen Clark responded in his opening statement that "this is not a trial about religion but a trial about science".

In 1968, the United States Supreme Court struck down as unconstitutional a state law forbidding the teaching of evolution on the grounds that it contradicted a literal interpretation of the Bible. This time the issue has been reversed. ACLU has brought the case on behalf of 23 local religious leaders, biology teachers and schoolchildren, claiming that the new law infringes their rights to freedom of religion.

"For practical purposes it is a tremendously important case for us because it is the first of its kind, and the decision of the court will get a lot of attention", said ACLU staff attorney Jack Novick before the case started. He pointed out that a similar bill has recently been passed in Louisiana, and that others are pending in up to twenty more states.

Three complaints are being made by ACLU. The principal charge is that the law violates the first amendment to the constitution. It is also being charged that the law infringes the academic freedom of teachers and schoolchildren, since it imposes conditions under which evolution can be taught; and that the language in the bill is unconstitutionally vague.

The state attorney-general in disputing each of these charges claims that, since there is nothing necessarily religious in the idea that the world came into being at the hands of some undefined form of creator, there is no problem with the constitution.

Each side will introduce a string of scientific witnesses to support its case. For ACLU, these include prominent academics in fields such as biology, geology and palaeontology; the state plans to introduces qualified scientists who claim their results substantiate the creationist theory of origins. Furthermore, unlike previous cases in which the relative merits of the two explanations were directly compared, this time the focus will be as much on the philosophy of science, with Judge William Overton deciding what types of ideas and reasoning can be described as "scientific" and "religious".

The case is expected to last for two weeks. If ACLU loses, it is already promising that it considers the case so important that it will take it as far as the Supreme Court, which has ultimate say on constitutional issues.

If the civil liberties group wins, then Mr Novik says it will press for full payment of its legal costs — calculated to be several hundred thousand dollars — against the state of Arkansas. But in that case, the state is expected to appeal against the verdict. So either way, the full legal debate is likely to last for a long time. David Dickson

reaching many of its conclusions makes it difficult to check on whether the agency is doing a good job. NRC member Mr Victor Gilinsky expressed concern that protection given in IAEA statutes to industrial, secret or other confidential information has been interpreted in the broadest manner to withhold information about the safeguards system.

Suggesting that undue secrecy made it difficult for NRC to fulfil its responsibilities for checking the adequacy of IAEA safeguards — responsibilities whose legal status still remains ambiguous — Mr Gilinsky said that the commission believed more information should be reported to IAEA's board of governors by its secretariat, and that the United States should try to obtain better information on overall international safeguards effectiveness and on any significant safeguards problems in countries receiving US exports.

Mr Kennedy replied that the general limitations on the agency's role — such as the substantial amount of classified information which it could not release — are "simply facts of international relations... The IAEA safeguards system entails a unique compromise of sovereign rights by many nations, and it is certainly no surprise that this compromise is subject to specific limitations". David Dickson

# Germany's breeder

# Gap unbridged

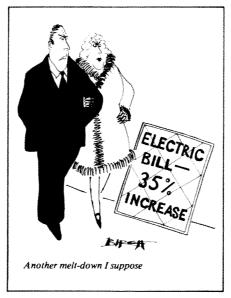
Ulm, West Germany

Rapidly rising costs are threatening the future of West Germany's SNR-300 fast-breeder reactor, with government and public service utility companies still arguing over who should foot the the increased bill if the project is to continue.

The Minister of Research and Technology, Andreas von Bülow, has succeeded in extracting from the utilities an increased financial contribution to the SNR-300 plant at Kalkar. At the end of October he announced that Rheinisch-Westfälisches Elektrizitätswerk (RWE), the leader of the owner/operator utility group, is willing to put up an additional DM 375 million. Two other utilities not so far involved in the project, Preussenelektra and Nordwestdeutsche Kraftwerke (NWK) are prepared to contribute DM 172 million.

Even so these pledges fill only half of a total shortfall of DM 1,100 million created by recent cost overruns. The federal government is unwilling to provide the extra funds from its own budget, and insists that the full amount should be paid by West German industry and in particular by the utilities, each financing a fraction corresponding to its share of electricity generation. "It is perfectly clear," von Bülow recently said, "that without an increase in the participation of the utilities, the future of the fast breeder is in danger and a halt to construction before the end of the year cannot be ruled out".

The weeks ahead will show whether von Bülow's warning is merely a tactical ploy or whether there is a real chance that work on the fast breeder will stop. Although budgetary problems have triggered off this latest confrontation, if the minister regarded the fast breeder as an absolute economic necessity it seems likely that he could accommodate the necessary additional DM 550 million over five years (DM 110 million a year) within this present budget - the ministry has a proposed total budget of DM 6,600 million for 1982 and an energy research budget of DM 2,550 million. "The present situation," he said, "necessitates a reconsideration of the original objectives of government support." At the same time the minister announced that government funds for microelectronics and biotechnology will be increased over the next few years.



Political uncertainty is one of the reasons why the utilities are reluctant to shoulder an increased share of SNR-300 costs. The Bundestag has reserved to itself the decision on whether the SNR-300 should be put into operation when completed. The utilities hesitate to commit themselves as long as this uncertainty persists, and the pledges made so far are contingent, among other conditions, on a positive decision from the Bundestag.

Licensing uncertainties have been another major obstacle – but here at least the greatest difficulties appear to have been overcome with the passing of the fourth part of the licence on 15 October.

Some utility companies originally argued that SNR-300 is a national project that should be financed from taxpayers' money. It remains to be seen whether they will soften their stand in the face of a threat to abandon the project. The West German utility association, VDEW, is now discussing alternative finance strategies including a research and development levy on electricity sales modelled on the present subsidy for West German coal used in the generation of electricity.

Otto Keck

# Israeli education

# More gloom

Tel Aviv

The complaint that cuts in higher education could seriously endanger Israel's scientific potential in the 1990s ran through the symposium organized at the end of November by the Israeli popular science magazine *Mada* to celebrate its twenty-fifth anniversary. Ironically, the symposium coincided with the *Israech* exhibition, a celebration of recent Israeli achievements in applied science.

While Isratech celebrated the success of Israel's funding policy, which for the past decade has emphasized applications, symposium participants maintained that, unless investment in higher education is stepped up, Israel's advantage in many sectors of technology could prove short-lived. The symposium was chaired by the physical chemist Dr Ephraim Katzir, formerly Katchalski, who produced the 1968 report which effectively switched government emphasis from pure to applied research.

Unlike other new states, Israel has a research infrastructure which antedates independence and which is based on institutions such as the Technion, the Weizmann Institute, the Hebrew University and the Volcani Agricultural Institute. Until the late 1960s, Israeli science policy was aimed at more of the same. The Kachalski report redirected government attention to the needs of industry. Only some of Katchalski's recommendations were implemented. Chief scientists were established in ministries involved in research and development, but the proposed upgrading of the National Council for Research and Development failed to materialize — indeed, in 1977, it was actually downgraded. being transferred from direct responsibility to the Prime Minister's Office to the new Ministry of Energy and Infrastructure. At the same time, all spending on research has been eroded by inflation, now 133 per cent a vear.

Even so, the post-Katchalski era has produced a number of achievements. Industry has done well, and now employs more science graduates. Agriculture has seen a steady stream of advances in crop breeding, soil chemistry, irrigation techniques and such novel methods as the sterilization of soils by solar energy. Both production and productivity have increased.

The symposium was chiefly alarmed by the other side of this coin – the continuing absence of a national science policy and the continuing brain-drain to the United States and Europe. The participants blamed people's willingness to leave on the cutbacks in university finance and the mismatch between what universities teach and what industry (and students) demands.

In the past ten years, there has also been a stagnation of student numbers in the exact sciences while total student numbers have soared. The underlying reason is not clear, although the decline has been greatest in the "traditional" fields such as civil engineering, while the most modern departments such as computer sciences are full to overflowing. This suggests that the universities are failing to adapt to modern trends — an ironic conclusion when they themselves have founded such institutions as the Institute of Forward Planning at Tel Aviv or the Neimann Institute at the Technion to act as potential policy advisers.

Employment problems are exacerbated by the relatively small payrolls of the new science-based industries. Would-be students therefore think twice before investing some five or seven years — delayed by a further three years military service — in an exact science when the job prospects are so uncertain.

The Jerusalem College of Technology, which was specifically founded to train the expert workforce the country needs, may be an exception. It is a relatively small and religious foundation where the all-male students study Judaism in the mornings and technical subjects to first degree level in the afternoons and evenings. The "new pioneering spirit" described by the founder and rector, Professor William Low, has presumably helped to take the edge off job uncertainty.

Perhaps inevitably, the symposium produced a gloomy forecast for the next couple of decades. According to Professor Joshua Yavner of Tel Aviv University, unless the government stops the cuts in higher education, Israel will suffer the loss of its main "natural resource" - educated manpower. Hitherto, the University Grants Committee, which administers the government's block funding of higher education, has tried to restrict the cuts to ancillary and non-academic fields (although library budgets have been considerably curtailed), but a point will soon be reached where no more such economies are possible. Then, inevitably, the output of science graduates and PhDs will begin to decline, and the new science-based industries, founded on the expertise of those who graduated in the 1960s and early 1970s, will fail to achieve their early Vera Rich promise.

# US research patents

# **Conflicts arise**

Washington

United States universities are lobbying hard in defence of their newly-won right to an automatic patent on all inventions arising from federally supported research. They are alarmed that their rights may be circumscribed in what may be the most profitable field — genetic engineering.

Two weeks ago, the House of Representatives Science and Technology Committee approved an amendment to a draft bill on patent rights, already approved by the Senate, to exempt the results of research using recombinant DNA techniques from

the broad provisions of patent legislation passed by Congress last December.

The amendment was offered by Representative Albert Gore of Tennessee, chairman of the committee's investigations and oversight subcommittee, and follows his criticism of an an agreement between Massachusetts General Hospital (MGH) and the German chemical company Hoechst that would, he claims, allow the company to benefit unfairly from research paid for by the US taxpayer (*Nature* 18 June, p.525).

Several Washington-based groups representing research universities have protested, saying that this amendment undermines the whole purpose of the new legislation, which is to speed the transfer of research results to private companies. They point out that three years ago, after consulting universities and industry, Dr Donald Fredrickson, then director of the National Institutes of Health (NIH), concluded that no special arrangements need be made for the application of patent laws to genetic engineering research.

An aide to Mr Gore said last week that the congressman might agree to withdraw the amendment before the draft bill reaches the floor of the House for discussion. The Senate has already passed such a bill which even expands the scope of last year's legislation to include not only universities and small businesses but all companies receiving federal research funds, and Senate aides said last week that they were totally opposed to the House committee's amendments.

However, Mr Gore still seems keen that the bill should make a sharp distinction between the control of research results funded from public and private funds. This could complicate efforts by NIH to interpret the new law's provisions on jointly funded projects.

Under the terms of the MGH/Hoechst agreement, the company will provide the hospital with up to \$50 million over a tenyear period to support basic research carried out in a new Department of Molecular Biology. All the research would be under the control of MGH and all research results could be freely published after a 30-day preview by the company, but Hoechst would have first refusal to license patentable results.

Both MGH and Hoechst insist that this arrangement is compatible with existing academic practice, that they have gone to great lengths to avoid any possible conflicts of interest or undue secrecy, and indeed that the terms of the agreement might act as a model for other universities seeking research support from the private sector.

Mr Gore, however, says that his concerns are endorsed by a report he commissioned from the General Accounting Office, the investigative arm of Congress, which pointed out ways in which the agreement might conflict with the patent legislation passed last year. Under the current legislation, any patents arising from jointly

funded research would remain the property of the research institution, but the federal government would be permitted free use of the patent, and would also be able to reclaim control of the patent rights. However, MGH acting director general Dr Joseph Martin insisted that a clear distinction would be made between research supported by the federal government and that financed by Hoechst.

Mr Gore remains unconvinced. He argues that maintaining a clear separation between separately-funded research projects is "simply a fiction" and promises that, even if his amendment to the patent legislation is dropped, he will be keeping a close eye on the MGH/Hoechst and other similar agreements, to protect what he describes as a massive public investment in recombinant DNA research. An official in the legislative office of NIH said last Friday that he felt the concern raised by the General Accounting Office; about jointly funded research "is certainly a problem that is not non-existent, although it does not currently appear very prevalent". A delegation from NIH will be visiting MGH next week to discuss the impact of the new patent legislation on future arrangements for federally funded research at the **David Dickson** 

# Science in France

# **Democratic** union

Grenoble

How far can democracy go? This question is emerging as one of those central to the new politics of science in France, and it was put to a remarkable test last week.

Professor Robert Tournier, the 47-yearold director of the renowned laboratory for very low temperatures at Grenoble, in the Rhônes-Alpes, announced some time ago to his colleagues that he wanted to resign the directorship to have more time for research in his own laboratory. Normally, the CNRS would take advice on a new director from the laboratory steering committee, and from the CNRS "parliament", the Comité National, and then would merely announce an appointment. This time it has another factor to take into account. Internal advisory committees in the laboratory could not agree on their recommendation for a successor, and Fournier, as a good democrat and socialist, asked the whole laboratory, including technicians, to give their advice on two candidates.

The resulting "election" took place last week. It has no official significance, and was nearly a draw. But the trades unions (their favoured candidate having won) are treating the event as a clear selection of director, and have written to the Paris headquarters of the Centre National de la Recherche Scientifique, to which the laboratory belongs, to say so.

As it happens, the narrowly-elected

candidate — a personable 39-year-old physicist called Daniel Thoulouze - is also likely to be the recommendation of the relevant section of the Comité National, so there may be no conflict. But the CNRS directorate is put in an awkward position whatever the result. If it selects Thoulouze, it will appear to approve of the grass-roots election of laboratory directors - which it wants to avoid. Yet if it selects another, it will appear to confirm the fears of the more radical scientists and the strong trades union movement in French science that, despite a socialist government, the selection of laboratory directors - who have great power over their laboratories will remain closed and centralized.

In the event, the CNRS may be forced to ask Tournier to continue. The new CNRS director-general, Jean-Jacques Payan, is not against elections in principle; but he says they should be undertaken only "in a very precise framework", meaning that the electorate should be appropriate to the category of post. But Payan is under great pressure from the grass roots, the technicians and the younger researchers, for a more democratic way of appointing directors, and he will certainly have to give in to some extent. As Tournier himself said this week, the question is whether the CNRS is forced into a system where directors must conform to majority opinion in a laboratory, or a system where the directors can be independent, but must subsequently seek to bring the whole laboratory behind them.

Meanwhile the unions of Grenoble, which have enjoyed a large degree of democracy (or at least, consultation), since 1968, are determined to be "serious" about the election. The candidates were grilled in debates, and their scientific and general laboratory policy examined, they said. The unions' objective is that the laboratory should continue to do good science, and they insist that that requires the director to have good relations with his staff, down to the lowest technician.

And to some extent, this policy is reflected at the Ministry of Research and Technology in Paris, which gave Payan his job at the CNRS. The strong feeling there is that science is more and more a collective exercise — and that democracy and efficiency go together. But on the other hand, they also feel at the ministry that too much democracy leads to "turbulence", a position which would seem to be close to Payan's own (which is perhaps not surprising, for the minister for science, Jean-Pierre Chevènement, and Payan have been close colleagues for a decade.)

In the end, the issue is going to be how far Chevenement is prepared to travel with the unions — and particularly the communist-affiliate CGT, which is strong at Grenoble. The CGT secretary here said last week that he and his union were watching and waiting. And if Chevenement does not come up to their expectations, he promises a struggle.

Robert Walgate

# What price IIASA?

Washington

The Vienna-based International Institute for Applied Systems Analysis (IIASA) has now been informed officially that the United States will be withdrawing its support for the institute from the beginning of 1983 (see *Nature* 3 December, p.390). This gives IIASA some breathing space in which to seek ways of keeping the United States involved — even if its contribution is severely reduced.

At present the United States and the Soviet Union together provide about half of IIASA's \$10 million annual budget, and the IIASA council is now looking at ways to reassess the dues from each of the member nations. At present the United States's \$2.5 million contribution comes from the National Science Foundation and is channelled through the National Academy of Sciences, and one possibility is that the money might be raised from other federal agencies. Private foundations are also being approached but so far all offers for support have been well below current levels.

Whether the United States will come back remains an open question. Founded in the early 1970s as a symbol of detente, IIASA has provided a useful window for both East and West on economic and social conditions on the other side — for example information about energy resources. But the institute has failed to stem doubts about the true academic value of its work.

**David Dickson** 

# Pakistan's space ambitions

# A military option?

Bangalore

Pakistan looks set to embark upon an ambitious programme of space research with clear hints that the spur is the prospect of military applications in the long term. Dr Salim Mahamood, chairman of Pakistan's Space and Upper Atmospheric Research Commission (SUPARCO), while unveiling the details of a 10-year national space programme in Islamabad recently, said that Pakistan did not want to be left behind in the space race, and that his country is studying in detail the configuration of a satellite which "can serve strategic purposes by taking pictures of military installations, army movements and acting as control, command and communication bases"

The setting up of SUPARCO earlier this year, with President Zia at its head, is said to have given fresh impetus to the hitherto slow-moving Pakistani space programme which is currently concerned mainly with launching sounding rockets from a base on the outskirts of Karachi. Dr Mahamood

claims that Pakistan is on the way to developing a communications satellite that "would be launched with the aid of either a European or an American rocket".

Since the successful flight test of India's SLV-3 rocket in July 1980, there have been fears in Pakistan that India might use experience gained with SLV-3 to build a strategic missile. Indeed, in November 1979 an Indian parliamentary committee was told that within six months of a political decision, SLV-3 could be modified into a medium-range ballistic missile. But the Indian Space Department stresses that military objectives are beyond the purview of the Indian space programme which is directed at "harnessing space for socio-economic development".

There is concern too that with the launch of the Bhaskara II satellite, India is developing the ability not only to survey the land for natural resources but also to carry out military surveillance over Pakistan.

Dr Mahamood claims that over the next five years, Pakistan will attempt to test a space launch vehicle of its own. The controversial deal between Pakistan and the West German private enterprise space agency OTRAG seems to be in obeyance. It is claimed that Colonel Gadaffi, OTRAG's host in Libya, had contacted Pakistan with an offer of OTRAG's technology.

How true is the rumour of a link between OTRAG and Pakistan is not known but some strategic analysts say that OTRAG could provide about 2,000 qualified space technologists badly needed to bolster Pakistan's space research efforts.

Meanwhile, Pakistan is negotiating with the US National Aeronautics and Space Administration for the setting up of a \$10 million ground station near Islamabad to receive earth resources data from the Landsat remote sensing satellite.

B. Radhakrishna Rao

# India's space programme

# Now for real

New Delhi

India entered a new phase in its development of a series of satellites for remote-sensing applications with the successful launching of its experimental satellite Bhaskara II, into a circular Earth orbit from the Soviet Union on 20 November. India's space programme for the 1980s includes as one of its two chief goals a major survey of natural resources. The other task is the development of space-based communications system. The first operational satellite, the National Satellite 1A will be launched in April 1982.

The 440-kilogramme Bhaskara II is an improved version of Bhaskara I, which went out of action, after 26 months, in August. Modifications to the camera system should eliminate the "corona discharge" which caused the breakdown of one of the two television cameras aboard Bhaskar I for a few months. And an extra

frequency has been added to its radiometer for more accurate measurements of atmospheric water content, a key factor in the satellite's subsidiary role of weather forecasting. The satellite also carries indigenously developed solar power cells for space qualification tests.

The next step after Bhaskara II will be the 600-kilogramme Indian Remote Sensing Satellite I (IRS I). A memorandum of understanding has been signed with the Soviet Union for launching it in 1986.

But India now has to face the prospect of a much more expensive space programme than in the past, since Bhaskara II marks the end of free launch vehicle and rocket booster facilities provided by the Soviet Union since the launch of the first Indian satellite in 1975.

Indian scientists are working on indigenous launch vehicles as alternatives, although experiments with the SLV-3 launch vehicle have so far achieved only qualified success. Nothing daunted, the Indian Space Research Organization is developing the Augmented Satellite Launch Vehicle (ASLV), based on SLV-3, which can put a 150-kilogramme satellites into space. India also plans to develop a polar satellite launch vehicle by 1986–87 to launch 600-kilogramme satellites into subsynchronous polar orbit 500 to 100 kilometres from the Earth. Sunil Saraf

# European environment

# One log-jam ends

Brussels

The Council of EEC Environmental Ministers which met in Brussels on 3 December was remarkably successful, with agreement being reached on several previously contentious issues.

The directive regulating discharges into the aquatic environment of mercury from the chlor-alkali industry was adopted. Attempts to push through the mercury directive and the "drins" directive (regulating aldrin, dieldrin and endrins) foundered on the dispute over whether quality objectives (favoured by the British) or limit values (preferred on the continent) should be used to assess the maximum permitted level of pollution discharged into water from an industrial plant. The ministers have now agreed on a clause requiring the latest technology to be used when building new plants.

The haggling over the Seveso directive on the prevention of major industrial accidents also ended at the meeting. The new French government has toned down its objections to the clause on the consultation of countries across the frontier from new plants. The consultations will now take place on a bilateral basis — without the involvement of the European Commission as originally proposed. Although the directive does not cover nuclear installations, the same consultation procedure is at the centre of a dispute between France and

# Electronic intrigue

Palo Alto

In probably the largest theft of semiconductor devices ever, \$2.7 million worth of integrated circuits disappeared over Thanksgiving weekend from Monolithic Memories Inc., a "Silicon Valley" company.

About 498,000 chips of two types were stolen. One kind, a programmable array, PAL, embodies circuits that provide a logic function in a wide range of computer products including video games, industrial equipment and computers used by the military. The second type, FIFO, is used to input data at one speed into computers and take it out at another.

About \$20 million worth of advanced computer chips disappear each year into a national and international underworld electronics trade. Integrated circuits are smaller than a fingernail, hard to identify and can sell for \$100 apiece. Many stolen chips are smuggled out of the United States into Eastern Europe. President Reagan's embargo on allowing Communist countries to buy high technology equipment provides a good market. Other chips are sold to pay narcotics dealers.

In 1979, an employee at INTEL stole 10,000 microcomputer devices worth \$1 million. They passed through blackmarket distributors in West Germany and eventually arrived at Siemens, a West German computer manufacturer. This robbery was traced when Siemens told INTEL that the untested chips were faulty. That the intensive electronic and human security at Monolithic Memories was bypassed suggests that in this case also the thief was an employee: the company is offering a reward of \$50,000 for information leading to an Charlotte K. Beyers arrest

Belgium. The French government intends to build power stations at Chooz, a few kilometres from the Belgian border.

Agreement was reached on a technical proposal to exchange data on sampling and monitoring levels of atmospheric pollution. However, the directive on safety levels for lead in air must wait until a communally acceptable method of monitoring atmospheric pollution is found.

Broad agreement was reached on the European Commission's third environmental action programme and its proposed policy to give environmental considerations greater weight when determining other EEC policies. Finally, despite much discussion, there was no decision to implement the Washington Convention—the EEC regulation on the trade in endangered species is, in fact, reported to be much tougher than the convention.

Jasper Becker

# Animal welfare

# Promise delayed

The new European legislation to protect laboratory animals seems to be in the hands of legislative snails. Optimists had expected agreement on the draft of a Council of Europe convention on "the protection of animals used for experimental purposes" last May. But the deadline passed, and the hope now is for agreement in March 1982.

Discussions have been under way since 1978, when the Council of Europe appointed an expert committee to draft model legislation. But the experts have so far failed to agree among themselves. Their latest disagreements relate to the "pain condition" that if an animal suffers severe and enduring pain, the experiment must stop and the animal be killed; the use of animals for more than one experiment; and the experimental procedures which should be included in the statistics on animal experiments.

Most of the representatives from the council's 21 member states object to the lack of provision for exemption from the pain condition in the latest draft. The exception is Britain, which wrote the draft when it took over chairmanship of the committee in April 1980. Britain argues that its own legislation, which includes the pain condition without exemption, has worked well since it was enacted in 1929.

Approval of the convention is also being held up by West Germany's objection to a clause which permits an animal used in one painful experiment to be used again. Most other countries accept the clause, some less willingly than others. A further stumbling block relates to whether animals used as controls, for example, should be included in statistics on animal experiments. Sweden believes that they should, but most other countries think their inclusion would push up the apparent number of experiments unncessarily.

The aim is to resolve these differences before the next annual meeting of the expert committee in March. When agreement is reached, however, a council of ministers will have to approve the draft before it can be laid before individual states for signature and ratification. Because ratification is a lengthy process, the convention is unlikely to come into force for several years.

Meanwhile, the European animal welfare lobby, increasingly vociferous, is asking for tighter controls than those in the latest draft. The Council of Europe's parliamentary assembly, which has no power but which can be influential, has agreed to discuss the lobby's demands and consider recommending changes to the terms of reference of the expert committee. If the assembly decides to recommend changes and the council of ministers agrees to implement them, the convention could be delayed indefinitely or even killed.

That, however, is unlikely. Most states

are eager for a convention as soon as possible, if only to head off the animal lobby's more extreme demands. Some, such as the United Kingdom, have already delayed national legislation pending a convention. Britain's Conservative government now has little more than two years in which to fulfill its last election promise to update the Cruelty to Animals Act of 1876. Two recent private members' bills to do just that have already come to grief. Judy Redfearn

# French reorganization

# Sector structure

Grenoble

Jean-Pierre Chevènement, Minister for Science and Technology in France, has finally decided on the structure and organization of his ministry. He has reaccumulated all the instruments and organizations of science and technology policy — dispersed since de Gaulle last had a major science ministry — and sorted them into a new structure.

The Délégation-Générale à la Recherche Scientifique et Technique, which acted as the relatively small office of the previous science minister, is to be disbanded and its staff re-absorbed. The same fate awaits the Délégation à l'Innovation et à la Technologie (which previously worked within the Ministry of Industry).

The new structure is claimed to be something midway between the highly dispersed "Rothschild" system of the United Kingdom in which individual ministries have control of large sums of research money which they can spend with research councils broadly as they wish, and the highly centralized German system; but the Chevènement structure is on the face of it considerably closer to the German than the British model.

The ministry will be divided into three sectors (plus the cabinet of personal advisers, which will be extended with the addition of a section to evaluate the progrees of his policies). There will be a "Direction de la Politique Générale", which, broadly speaking, will administer basic science through the "grands organisms" (the Centre National de la Recherche etc); a "Direction du Développement Scientifique et Technique et de l'Innovation" which will actively promote contacts between science and industry and mastermind the scientific renovation of the French economy (the matter closest to Chevenement's heart); and a high-flying "Mission Scientifique et Technique". This body, headed by Yves Farges, a one-time solid-state physicist and the principal advocate for a European synchrotron radiation source, will act as a kind of interface between the other two sectors and the minister himself.

The staff of the ministry at present numbers some 250. By the time the changes are complete — and the ministry has moved to a new location on the Montagne Ste-

Geneviève (the old site of the Ecole Polytechnique) the staff will probably have grown to 400.

Robert Walgate

# Scientific fraud

# In Bristol now

A further case of falsification in the scientific literature has come to light. The following letter has been received from Dr M. J. Purves of the Department of Physiology at the University of Bristol:

SIR — I very much regret to have to report that data published in the preceedings of the 28th International Congress of Physiological Sciences (Purves, M.J. 1981 Cerebral Blood Flow and Metabolism in the Sheep Fetus. Advances in Physiological Sciences 9: 199; 126. Pergamon, Oxford & Adademiai Kiado, Budapest) are false. I must also emphasize that none of my colleagues was involved in the preparation of this paper and the responsibility was mine alone.

Bristol, UK

M.J. PURVES

Dr Purves, a reader at the University of Bristol, resigned his post with effect from 1 November after an internal investigation.

The paper concerned describes the use of recently developed techniques for investigating the function of the mammalian (sheep) brain in utero. The article describes the use of 5-deoxyglucose as an index of metabolic activity in central nervous metabolism. One of the objectives of the study was to demonstrate that 5-deoxyglucose (not metabolized in the usual way) is taken up more slowly by the fetal mammalian brain during periods when the embryo is asleep.

The University of Bristol seems to have acted quickly since some of Dr Purves's junior colleagues drew attention to the irreproducible features of his published paper earlier in the year. An agreed announcement of the circumstances was delayed for family reasons. Dr Purves said earlier this week that the falsifications consist of the data published. His senior colleagues say that they cannot understand why such a talented person, well-supported by the Wellcome Foundation and the Public Health Services, should have followed such a course.

# Erratum

Nature must apologise to one or other (or both) of Sir Andrew Huxley (President of the Royal Society) and Professor T.R.E. Southwood that the latter's photograph appeared last week in place of the former's. The Royal Society's anniversary celebrations this year appear to have been especially prone to accident — the President of the Fellowship of Engineering, half-way through an impassioned speech on the importance of engineering, referred to the present President's predecessor-but-one, Dr.A.L. Hodgkin, as Professor Hodgkinson.

# Primitive life in France

Paris

The life and times of Europe's earliest inhabitants form the focus of a new exhibition at the Musée de l'Homme in Paris. The exhibition, which opened on 9 December and will run until April 1983, illustrates major advances in human development over more than a million years of European prehistory.

Evidence of human presence beginning with what are reputed to be the oldest stone tools in Europe is traced up to the appearance of fully evolved Neanderthals some 125,000 years ago, Remains from more than 100 sites in 13 European countries are on view, combined with replica living floors and two reconstructions of palaeolithic shelters.

The main advance portrayed in the exhibition, according to Professor Henry de Lumley, exhibition organizer and director of the Laboratory of Prehistory and the museum, is the domestication of fire in Europe some 400,000 years ago. By 300,000 years ago distinct cultures throughout Europe possessed knowledge of fire, were making various tool assemblages, living in organized encampments and hunting in groups.

By this time too, early Europeans had apparently begun to construct more elaborate living shelters with several distinct areas. The remains of two such living floors from sites excavated by Professor de Lumley provide the basis for the striking hypothetical recreations of a hut from Terra Amata and a cave shelter at Le Lazeret (both near Nice in France).



The Terra Amata hut is built from wood poles and pine branches on a replica rocky Mediterranean shore and is based on remains dated at about 380,000 years ago. The Le Lazaret cave shelter suggests an even more comfortable lifestyle by about 130,000 years ago.

Specialists may well debate the interpretation of the stones, bones, dates and reconstructions in this exhibition, but all visitors will go away with a lasting impression of how prehistorians recreate the past and what daily life might have been like for Europe's earliest inhabitants.

Richard Dreiman

# CORRESPONI

# Psychological slip

SIR - Reviewing H.B. Gibson's biography of Hans Eysenck (Nature 5 November, p.44) P.E. Bryant writes: "He (Eysenck), more than anyone, shaped the development of clinical psychology in this country;

This statement may be misleading to a readership as diverse as Nature's. In this context the term "clinical psychology" is used, or perhaps linguistically corrupted, to signify the application of behaviouristic or learning theories to the treatment of psychological disorders. It does not refer to the application of psychological theories collectively to the treatment of such disorders, which is the rational inference.

It may well be the case that Evsenck has shaped the development of behaviouristic clinical psychology in this country. However, it would clearly be incorrect to suggest that he has had much influence on the development of certain other principal approaches to clinical psychology such as psychoanalysis.

This duality of meaning is a potential source of considerable confusion, but one which can be simply obviated by qualifying the specific school of clinical psychology referred to.

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# **Species difference**

SIR - There is a serious misunderstanding which is impeding any resolution to the current debate on macroevolution. Recent analysts of the fossil record report a pattern which they claim "strongly supports the notion that speciation is a qualitatively different phenomenon from gradual, intraspecific microevolutionary change"1. This is true. Others point out that the observed details of fossil history "do not force us to change our views on the genetic mechanisms of the origin of species"<sup>2</sup>. This is also true. The misunderstanding lies in the idea that they are referring to the same thing, for they are not. They are addressing speciation on two entirely different levels.

The model of evolution in contention is the 'punctuated equilibrium'' proposal of Eldredge and Gould<sup>3</sup>. Evolution at the specific level is seen as occurring in short rapid bursts followed by relatively long periods of evolutionary non-change or stasis. The words "short" and "long" are used in the context of geological time. That is, morphological changes associated with the transition from an old species to a new one may occur on the order of 103 to 104 years, which is recorded as an instant in most fossil assemblages. Those who experiment on the evolutionary genetics of living organisms are quite correct in maintaining that this is more than enough time for classical selection mechanisms to have conceivably produced the observed changes. This much is clear to proponents of punctuated equilibrium as well. Why then the call for qualitatively new explanations?

The mechanisms depicting "how" new

species arise have in the past been considered explanations of "why" they develop as well. New species are classically described as being the logical result of long, slow, continuous selective pressure. This continual change is the view of phyletic gradualism. If, however, the prevailing pattern is one of stable equilibrium only punctuated with brief periods of evolutionary change then the mechanism of that change does not tell the whole story. The phenomenon of stasis, not the sudden appearance of new species, is the true source of the conflict with the traditional evolutionary school of thought.

Matters are not reconciled by appealing to selective pressures varying dramatically over time. The environment is dynamic and in a constant state of flux. While some periods may be characterized by a less stable climate than others, there are always marginal environments and isolated communities within which evolution can work. In addition the fossil record does not support the contention that novel stable species arise and replace parental populations only at times of the most extraordinary environmental instability. That is not to say that new species do not arise at times of climatic shifts, but other shifts of equal magnitude may not result in the establishment of new species while novel forms may invade from a marginal locale and become dominant at times of overall relative environmental tranquillity. Thus classical Darwinian theory does not provide an explanation for the punctuated pattern of speciation now emerging from the fossil

The models of population genetics may be perfectly adequate at the fine level of how new genetic entities are formed but they do not address questions at the grosser level of the overall patterns. New qualitatively different schemes are therefore needed. Conversely, while theories of a qualitatively different sort are indeed required by the recent reevaluations of the fossil record, the classical genetic mechanisms of speciation are nonetheless compatible with punctual equilibrium.

CLIFF TABIN

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# Question of faiths

SIR - It is admirable to see scientists such as A.H. Batten and E.W. MacKie (Nature 30 July, p.402) come to the aid of the theory of evolution when they perceive that it is threatened by creationism. Such enthusiasm shows their dedication to rational thought. However, when in their eagerness they instead misrepresent its basic scientific tenets one has to wonder just whose side they are on.

Batten is quite correct in criticizing neo-Darwinian evolutionists for being overly dogmatic and thus in fact hastening the rise of the creationist movement. I cannot accept, however, the assertion that the theory of

evolution (that is, descent with modification) is the same as the theory of natural selection and, therefore, if chance mutations are found to be insufficient agents of phylogenetic change one must look for a purposive element to explain the observed hierarchical order in nature. This self-imposed dichotomy leads Batten to reject Popper's critical rationalism for Kuhn's paradigm of "normal science"

We are thus asked to console ourselves with knowing that if we wish to avoid a dogmatic assertion of evolution as a fact then it is necessary to see it as simply the best explanation we have at the moment. Since we must choose between neo-Darwinism and creationism there is not much chance of ever escaping the bonds of the existing paradigm. Batten's complacency with "normal science" and inability to consider other, natural causative agents of evolutionary change (of which the literature is full) only serve to weaken any intended appeal to reason. The arguments presented are also a serious misrepresentation of Popper's philosophy.

MacKie, on the other hand, has chosen to ignore Popper or any other proponent of the hypothetico-deductive method altogether (ignorance of this concept is difficult to believe) and take us almost 400 years into the past. The notion of science as inductive and mythology as deductive might have impressed Francis Bacon, but it has no place in modern thought. Someone should tell MacKie about David Hume. The only way out of the conundrum is to realize that science arises from grand, explanatory ideas, ranging from the metaphysical to the conjectural, and it is only after this initial synthesis that rigid testing is invoked. It is the creationists' uncritical appraisal of their own theories, in both testing procedures and methods of formulation, that marks their endeavours as unscientific. The inductive-deductive barrier has nothing to do

Both authors should learn to live with Popper's observation that rational thought is based on irrational faith in reason. I would add that evolutionary biology is similarly based on a faith that the natural causes we observe are indeed natural causes, and not the manifestations of a supernatural being. We are, then, faced with two faiths: those of rationality and irrationality. The former we can modify with critical argument, the latter we cannot. Yet, just because rationality arises from an irrational faith, we are not compelled to consider every aspiring scientific argument on equal grounds. It is not a case of the 'anything goes" attitude of relativism. I believe that is what MacKie worried about as egalitarianism. There should be no problem, since every hypothesis must prove itself in its explanatory power, testability and predictive success. Thus, as MacKie notes, the acceptance procedure for hypotheses must be shamelessly elitist (with respect to the particular hypothesis, not its proponent). Anything less would be uncritical and therefore unscientific. Faith per se is no threat to science; dogma and woolly-mindedness are.

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105s-1) to account for both the NMR and ESR observations<sup>11,12</sup>

Reconstitution studies allow the experimentalist to manipulate the magnitude of the lipid-protein interaction by varying the relative concentrations of lipid and protein in the membrane. NMR studies of reconstituted membranes were described which indicate that as the protein concentration increases, moderate (about 20 per cent) decreases occur both in the average order (through deuterium order parameter measurements3) and in the rate of lipid chain motion (from T, relaxation measurements in proton1 and deuterium3 studies). An analysis of both the ESR spectral components shows that the rate of lipid chain motion for boundary and nonboundary lipids is decreased by the protein, when compared with the non-proteincontaining membrane. Unfortunately, no conclusive information on the amplitude of chain motion can be deduced from these spectra<sup>5</sup>. ESR and NMR observations therefore imply that the 'rough' protein interface both disorders the acyl chains interacting with it and restricts their motion. The magnitude of disordering or motional inhibition again depends on the spectroscopic method being used. These data appear consistent with the idea that proteins induce a rather specific perturbation on the membrane lipids, disordering and motional restriction, unlike the effect of temperature which decreases both the rate and amplitude (together often called 'fluidity') of acyl chain motion.

At the meeting, the relevance of dynamic lipid-protein interactions was questioned during a discussion period by the interjection "So what. . .?". During this and subsequent lively interchanges, it was suggested that lipids at the protein interface (1) may be important for efficient sealing of the bilayer to the protein, particularly vital in transport processes; (2) provide a motional buffer between the peptide backbone to the bulk membrane lipids; (3) sterically maintain at equilibrium the active conformation of a membranebound enzyme, which would require a fast motional interaction on the biochemical time scale defined by the catalytic turnover rate of  $10^2-10^3$ s<sup>-1</sup>; and (4) are able to contort and conform to the protein interface to solvate it in a way that minimizes the energy configuration within the whole membrane.

# New low temperature phases of H<sub>2</sub> and D<sub>2</sub> at ultrahigh pressure

from J.C. Raich and R.D. Etters

AT very low temperatures and atmospheric pressure the molecules in solid parahydrogen (p-H<sub>2</sub>) and orthodeuterium (o-D<sub>2</sub>) are in spherically symmetric rotational states. At a sufficiently high density it is expected that the interactions between the hydrogen molecules deform the average molecular density distributions so that the molecules spontaneously align in an orientationally ordered state. At this transition, the essentially free molecular rotations change to hindered rotations or librations about equilibrium orientations. These equilibrium orientations depend on the crystal structure of the high pressure phases which probably differ from the hexagonal close-packed low pressure structure. This rotation-libration transition in the hydrogens was first predicted by Raich and Etters1 using a mean field theory. Subsequently mean field calculations<sup>2</sup> and Monte Carlo simulations<sup>3</sup> have supported the first-order nature of the transitions as well as the approximate density dependence. These theories predict critical pressures at roughly 75 kbar for o-D, and 270 kbar for p-H, using an extrapolated equation of state4.

Recent improvements in high pressure techniques, made possible by the development of diamond anvil cells, have renewed interest in the rotation-libration transition and the resulting prediction of new low temperature, ultrahigh pressure, orientationally ordered phases of the solid hydrogens. Indirect evidence of such a transition in hydrogen has recently been reported from the pressure dependence of Raman measurements of the H-H vibrational stretching band  $Q_1(1)$  in hydrogen by Sharma, Mao and Bell4. The notion that changes in the molecular state of solid hydrogen begin to occur at approximately 200 kbar is supported by the flattening of the curve of the frequency shift  $\Delta\nu[Q_1(1)]$ versus pressure which begins to occur at 200 kbar. The decrease in the half-width of  $Q_1(1)$  at about 175 kbar is taken as additional evidence for a lessening of molecular rotation. The further flattening of  $\Delta\nu[Q_1(1)]$  at higher pressures can be interpreted as an indication that molecular rotation has ceased for pressures exceeding 375 kbar. Sharma et al.4 also observed that bands attributed to molecular rotation become diffuse and disappear well below 375 kbar at 298K. However, to attribute the observed changes in the vibrational mode to changes in the rotational motion is only one of several possible explanations. Another possibility might be a pressureinduced change in the electronic charge distribution of the molecule.

Recently, Lagendijk and Silvera<sup>5</sup> have

studied the rotation-libration transition, taking a different theoretical approach. They investigated the predicted behaviour of the collective rotational excitations in the solid as a function of pressure, and found that increasing the density of the solid leads to a softening of the collective rotational excitations with rotational quantum number J = 2. The excitation energy of these J = 2 rotons approaches zero at the X boundary point of the Brillouin zone of the face-centred cubic structure for which the calculations were performed. The existence of this roton instability requires a symmetry breaking of the orientational ground state with J=0. resulting in an ordered structure in which the orientational alignment occurs along the <111> directions of the face-centred cubic lattice. This approach predicts roughly the same transition densities as the earlier mean field treatments. On the other hand, recent Monte Carlo calculations by Aviram, Goshen and Thieberger, using the full anisotropic interaction between H, molecules, seem to predict transition densities which are about 60 per cent higher than the mean field densities6

Direct experimental evidence of the existence of a new high pressure phase of o-D, and the precursor of a similar phase of p-H<sub>2</sub> has recently been reported by Silvera and Wijngaarden using Raman scattering which has proved to be a very effective probe of the solid hydrogens. In the low pressure hexagonal close-packed phase, the Raman transitions of interest are three distinct  $J = 0 \rightarrow J = 2$  transitions. In the predicted high pressure phase, the crystal structure is probably different, with the result that the corresponding rotational excitation spectrum will be changed significantly. In o-D, at about 150 kbar, the J = 2 rotons begin to broaden and turn down in frequency. Between 200 and 278 kbar the roton spectrum coalesces into a single broad rotational band. Above 278 kbar the rotational band splits abruptly. These changes are accompanied by a sharp shift of the  $v = 0 \rightarrow v = 1$  or  $Q_1(0)$  vibrational frequency. The same change in behaviour is observed when the temperature is lowered below the critical temperature. These changes are considered to be the manifestation of a phase transition to an ordered phase at a temperature T = 5K and pressures  $p \ge 278$ ± 5 kbar in o-D2. Although no strong characteristic of a first-order transition was observed, it was not possible to rule out a

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simultaneous hexagonal close-packed to face-centred cubic structural change analogous to the case of the zero-pressure o-H<sub>2</sub> and p-D<sub>2</sub> orientational transition.

 $\ln p - H_2$  for p > 500 kbar, the solid appears to be approaching the region where a similar phase transition might occur. However, higher densities seem still to be required. It is likely, however, that the rotation-libration transition in p-H, will occur before the metal-insulator transition.

The observed transition pressures, 278 kbar for o-D2 and in excess of 500 kbar for p-H<sub>2</sub>, are much larger than the theoretical predictions. Whether this difference is due to the approximations made in the various theoretical approaches, or to an inaccurate representation of the intermolecular potential, remains to be elucidated. In particular, the use of asymptotic forms for the anisotropic contributions to the H,-H, interaction potential at high pressures should be questioned seriously. A comparison of the results of Silvera and Wijngaarden8 with those of Sharma, Mao and Bell may be difficult as the latter started with fluid hydrogen at room

temperature and the former with p-H, at only a few degrees Kelvin.

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# New pathways for chlorinated dioxins

from Alistair Hay

UNTIL recently many would have argued that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) — a toxic contaminant produced in the manufacture of 2,4,5-trichlorophenol — was not metabolized in animals. The chemical seemed to require no metabolic activation to make it more toxic and did not appear to be metabolized before excretion.

But times have changed, and so has the evidence. It is still true that TCDD is active in its own right, but it is equally clear that some metabolism of the chemical occurs in animals. Poiger and Schlatter (Nature 281, 706; 1979) were the first to report that TCDD was hydroxylated in the rat. Others are reporting similar findings. At a recent workshop\* R.A. Neal (US Chemical Industry Institute of Toxicology) reported that Golden Syrian hampster hepatocytes will metabolize TCDD in vitro, producing a pattern of metabolites similar to those excreted in the bile and urine of the animal.

The faeces (bile) is the major route of excretion in the rat, hampster and mouse. The TCDD which is retained in tissues after administration appears to be unmetabolized. For the small amount of the chemical which is metabolized - generally of the order of one per cent — the process appears to begin with hydroxylation to phenols. The hydroxyl groups, Neal suggests, are then converted to. glucuronides prior to excretion.

Before this reaction scheme can be proved, the metabolites will have to be positively identified. H. Poiger and H.R. Buser (University of Zurich and Swiss Federal Research Station respectively) have identified metabolites in the bile of the rat and the dog which enables them to suggest that in these two animals, TCDD is metabolized by the reaction scheme shown in Fig.1. They claim that metabolism of TCDD is a process of detoxification. Extracting the metabolites from the bile of dogs and testing for toxicity in the guinea pig shows them to be at least 100 times less toxic than the TCDD itself.

According to S. Safe (Texas A & M University) there are common features in the metabolism of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). With all these chemicals the rate of metabolism decreases as the degree of chlorination increases; and the major metabolites formed are phenolic compounds which are generally less toxic than their parent hydrocarbon precursors.

When aromatic hydrocarbons reach a certain size and lipophilicity it seems that they are poorly retained in the body. There is considerable species variation in the metabolism of different isomers of these compounds and lipid stores have a major role in the regulation. H.B. Matthews (NIEHS, Research Triangle Park, US) reported that animals eventually reach a state of dynamic equilibrium with adipose and other tissues playing their part in sustaining blood TCDD concentrations. Should the blood concentration fall, dioxin will move out of other tissues and back into

the blood in sufficient amounts to restore the equilibrium. The process, says Matthews, is not rapid, but occurs over a period of days, perhaps even as long as a

When animals are starved TCDD is mobilized from adipose tissue and this could lead to blood dioxin levels reaching concentrations which ultimately prove toxic. Fortunately, in human subjects this situation is unlikely to occur. The body burden for TCDD in humans is thought to be low. But low as blood levels of this chemical are, it is becoming clear that it can stay around for a long time.

C. Rappe (University of Umea, Sweden) reported that pentachlorodibenzofurans could be detected in the liver of a young Japanese man — at a level of 1.2 ng g-1 -12 years after he was exposed to the chemicals. The PCDFs were present as contaminants in PCBs which affected a batch of cooking oil in Japan in 1968. The

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RAT DOG TCDD Dechlorinated metabolite not yet determined Оон (OH), Fig. 1 Metabolism of TCDD in the rat and dog.

<sup>\*</sup>The 'International Symposium on Chlorinated Dioxins and Related Compounds' was held at the Sheraton National Hotel, Arlington, Virginia, 25-29 October 1981.

victims affected by this oil are known as the 'Yusho I' cases. A similar 'Yusho II' event occurred in Taiwan in 1979.

It is currently standard practice to assess exposure to chlorophenols by measuring the levels of these chemicals in urine. Rappe, however, suggests that blood measurement of the PCDF and PCDD contaminants present in the chlorinated phenols is a superior method. Urine concentrations of chlorinated phenols fall rapidly to undetectable levels in a very short time whereas PCDDs and PCDFs can be detected in blood years after exposure. Although the significance of TCDD levels in blood is not yet clear, many believe that it is important to have more of these measurements to try and relate blood levels to clinical symptoms.

It is a pity this information is not available now for assessing the carcinogenic risk from TCDD. Reports from Sweden (Hardell and Sandstrom Br. J. Cancer 39, 711: 1979) suggest that forestry workers exposed to chlorinated phenols and the herbicide 2,4,5-T have a sixfold higher incidence of soft tissue sarcomas. A repeat of the investigation by one of the Swedish scientists to take account of some criticisms of the earlier study confirms the initial findings (Hardell Scand. J. Work envir. Hlth 7, 119; 1981).

However, studies conducted in other countries have so far failed to confirm Hardell's reports. A cohort of herbicide applicators in Finland exposed to phenoxy herbicides for at least two weeks between 1955 and 1971 have been monitored since 1972. The incidence of tumours in this group is no higher than would be expected and no cases of soft tissue sarcoma have been observed. Similarly, preliminary findings from a study in New Zealand, reported by A.H. Smith (Wellington Hospital), in which soft tissue sarcoma cases were compared with patients with other forms of cancer showed that the individual's place of work bore no relationship to the type of tumour seen.

The different findings from the various studies are disturbing. So much so that a workshop study group on the health implications of TCDD exposure concluded that it was a matter of urgency that additional studies be done to determine whether there is indeed a causal relationship between soft tissue sarcoma and exposure to phenoxy herbicides such as 2,4,5-T.

Experimental design of the epidemiological studies may have some bearing on the different results obtained. If so, it is important that new studies should take account of this. There is general agreement that all the studies performed to assess the carcinogenic risk from exposure to TCDD and phenoxy herbicides were well designed. However, it is felt that some studies may have bias which has so far gone unrecognized. If this is the case it will be some time yet before there is unequivocal evidence about the health risk from exposure to 2,4,5-T.

# Parasites affect behaviour of mice

from F.E.G. Cox

PARASTTIC WORMS are ubiquitous in small mammals, even in many laboratory colonies, and are almost invariably ignored in behavioural studies. Two recent investigations indicate, however, that they may markedly influence both the acquisition of dominance and general exploratory behaviour of mice.

When male mice are reared in isolation and then put together fighting occurs and a hierarchy of dominance is established with one dominant male. This simple procedure has allowed an Australian scientist, W.J. Freeland, to investigate the effects of nematode infections on the behaviour of mice!. Using groups of two infected and one uninfected mice, he found that those given 50 or 150 larvae of Nematospiroides dubius became dominant as often as uninfected ones but those given 250 larvae became dominant less frequently than would be expected. Once dominance in uninfected mice was established, however, a subsequent infection with 250 larvae did not lead to its loss. Freeland argues that, as dominance may be related to mating success, infected mice are at a reproductive disadvantage and that females mating with uninfected or lightly infected males protect themselves and their offspring from infection and also select males with genes for resistance to

Even though these results are convincing and the conclusions self-evident, they are subject to a number of reservations. First, the number of mice used in each trial, three, was small, although 20-30 trials were used for each level of larval infestation; a more satisfactory procedure would have been to use 6-10 mice. Second, the numbers of worms that brought about behavioural changes were relatively large. In natural infections the majority of mice carry small numbers of worms and very few carry large numbers2 and 250 is more than would normally be expected. The reasoning also fails to take into account the true nature of nematode infections in mice in the wild for the female is as likely to become infected from another male as from her mate as there is no permanent pair-bonding and, as rodent territories are not absolute, males tend to roam over large areas.

The suggestion that female mice might effectively select for genes for resistance is also open to question. Although the importance of genetic factors in resistance to nematodes in general and to N. dubius in particular is well documented3, individual animals acquire their initial infections with large and small numbers of worms purely by the

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chance ingestion of infective stages and only later mount an immune response; so in primary infections, like the ones described, the female would be selecting for luck rather than resistance. Furthermore, more active males range further and thus have more opportunities to become infected; this is well known for ectoparasites such as ticks4 and is presumably also true for worms of various kinds.

The second set of experiments were carried out in the United States at the State University of New York and Cornell<sup>5</sup>. In these experiments, mice were infected with the dog nematode Toxocora canis. The larvae of this worm cause considerable damage by burrowing about the body of abnormal hosts and may cause blindness in children. In mice, the larvae may enter the nervous system and brain. The American workers infected mice with T. cants and subjected them to a number of behavioural tests to assess such activities as exploratory behaviour, areas traversed, avoidance reactions and spatial discrimination in mazes. The various tests were carried out sequentially over a period of 115 days and in all tests infected mice did less well than infected ones. The authors conclude that T. canis infection reduces exploratory activity, learning and motor coordination. These results are easier to understand than those of the dominance experiments for the mice infected with T. can's were all found to have parasites in the brain at autopsy, but a direct correlation between brain damage and behavioural changes has not yet been established.

These two sets of experiments taken together, however, do suggest that parasites can modify the behaviour of rodents and although this is well known in other hosts the gross behavioural changes are usually brought about by physical damage such as is caused when a parasite lodges in the eye or brain or occupies a considerable amount of the body cavity. The interactions between parasite and host may, however, be more subtle than this and parasites in the gut or kidney may affect the odours produced by the host or may even affect its hormone balance. It is likely that parasites will be increasingly implicated in alterations in host behaviour; however the nature of these changes will not be resolved by the use of simple behavioural experiments but will have to follow the advances being made in our understanding of the underlying mechanisms of animal behaviour. П

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# Useful tropical legumes

from Robert M. May

AMONG the many potential applications of research on recombinant DNA that are exciting commercial enthusiasm is the hope that cereal and other crops may be genetically engineered to fix their own nitrogen. This is a consummation devoutly to be wished, because current trends in the cost of energy are making nitrogenous fertilizers extremely expensive for peasant farmers. At the same time, however, there appear to be many plants in the family Leguminosae that are greatly underexploited; some are extensively used in one locality but unheard of elsewhere, while others are virtually unknown but have particular attributes that suggest they could be developed into major crops. The legumes provide their own nitrogenous fertilizer through bacteria that live in nodules on the roots of the plant, where the bacteria convert nitrogen gas from the air into soluble compounds the plant can absorb and use. Although a few other plant families include species with this ability, legumes produce the bulk of biologically fixed nitrogen. Even today, the total nitrogen added to the world's soil by cultivated leguminous crops exceeds that added by fertilizers.

Leguminous plants are found throughout the world (including a few aquatic legumes), but the greatest variety is in the tropics and subtropics. There are approximately 650 genera and 18,000 species of Leguminosae, which makes them the third largest family of flowering plants (after the Compositae and the Orchidaceae). The family is divided into three subfamilies: the Caesalpinioideae, comprising about 2,800 species which are mainly trees of the tropical savannas and forests of Africa, South America and Asia; the Mimosoideae, again comprising about 2,800 species, which are mostly small trees and shrubs of semi-arid tropical and subtropical regions of Africa, North and South America, and Australia (Acacia species are prominent in this category); and the Papilionoideae, comprising around 12,000 species, which are mainly herbs and are distributed worldwide. Legumes can most easily be recognized by the pods they bear; these pods come in an enormous variety of sizes and shapes, from one not much bigger than a pinhead to one the size of a tennis ball, and they contain from one to several dozen seeds.

A recent study (Tropical Legumes: Resources for the Future, National Academy of Sciences, Washington, DC, 1981), emphasizes that "Of the thousands of known legume species, less than 20 are used extensively today". Cultivation is most commonly for the seeds (called beans, grain legumes, or 'pulses'), which rank

second only to cereals as a source of human and other animal food. Nutritionally, leguminous seeds are typically two to three times richer in protein than are cereals. Before the advent of the potato in Europe, beans constituted much of the diet of the poorer classes. Today, they remain a major food in Latin America, on the Indian subcontinent (especially lentils and chickpeas) and in the Far East (especially soybeans). Greater emphasis on pulses, in contrast to the 'Green Revolution's' emphasis on cereals, could do much to alleviate the chronic protein deficiency found in many developing countries.

The NAS report notes many promising pulses that stand in need of further research. The virtues of the Winged Bean have been extolled earlier (Nature 266; 590, 1977). The Bambara Groundnut is an African pulse which seems to constitute a complete food: although "its seeds have less oil and protein than peanuts, they do have more carbohydrates and make a well balanced food with a calorific value equal to that of a high-quality cereal grain. .. it can thrive in arid inferior soils where peanuts fail, it resists pests and diseases, and, if managed well, can give high yields". Another example is the Moth Bean, which is "an exceptionally hardy South Asian legume that thrives in hot, dry, tropical conditions, [producing] nutritious seeds and green pods, leafy forage for hay or pasture, and a soilbuilding 'living mulch' to complement orchard crops and to protect and improve fallow land. . . . It is likely to prove very useful in extending agriculture production into marginal regions - especially those bordering tropical arid zones". An interesting candidate for a cash crop is the Yeeb, from the Horn of Africa, which flourishes in tropical arid zones and produces seeds with taste akin to macadamia and pistachio nuts.

In earlier centuries, when natural dyes were the only way to colour fabrics, legumes played a key part in the history of Western exploration and colonial development. The blue dye indigo, extracted from small leguminous shrubs of the genus *Indigofera*, was a major product of India, traded eastwards to China and westwards to Europe. More valued than spices, it was a major reason for establishing Portuguese, Dutch and British colonies in India. In South America, the small leguminous tree *Caesalpinia echinata* vielded a wine-red essentially identical to

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bresil (from Caesalpinia sappan), a dye that Europe had been importing from Asia for centuries. This dye soon became the most important export from the new land to Portugal, whence the merchants became known as bresilieros, and the region as Brasil or Brazil.

Turning from the past to the future, the NAS report systematically lists many kinds of uses to which legumes may be put, beyond the relatively familiar pulses.

Native tribes in several rural areas of the world eat tubers harvested from wild or cultivated legumes. Most of the species so used have not been subjected to scientific research, but a few that have been analysed show a remarkably high protein content (sometimes several times higher than the root crops in conventional use in the tropics). The NAS report urges the study of these little known root crops, with a view to improving and disseminating the most productive and nutritious ones.

There are several interesting fruits that could be more widely exploited. For instance. Mediterranean peoples have for millenia enjoyed the sugar-rich, mealy pulp of carob pods. "The handsome, droughttolerant carob tree deserves more research and widespread exploitation in semi-arid areas, for in addition to pulp it provides a chocolate substitute, high-protein flour and an industrial gum, as well as shade, beautification, erosion control and forage." Although in common use for centuries, the tamarind remains largely unimproved and little cultivated; up to half the mass of a tamarind pod is sour-sweet pulp, which is used in refreshing drinks (and in Worcestershire sauce). The honeylocust can be easily grown from seed, suckers, or cuttings, and grows fairly fast to produce giant pods from the fourth or fifth year. The sweet, succulent pulp of the pods is relished by humans and other animals. As I have one in the backyard, I learn with mixed feelings that it is one of the hardiest, most adaptable and most generally useful fodder trees known, but that care is needed in new locations as it can form dense thickets and become a pest (as it has in Queensland).

Too often, woody plants are overlooked in programmes of research for improved forage in tropical regions. Yet, as the NAS panel notes, browse shrubs and trees can complement herbaceous pasture species and can be crucial to the survival of animals during droughts (when shallow-rooted species often shrivel to straw). Emphasizing that many species undoubtedly await discovery and exploitation, the NAS study focuses on the thorny, flat-topped Acacia tortilis bush (one of the most drought-tolerant trees of semi-arid areas of Africa and the Middle East, and a great soil stabilizer) and on other species of Acacia and Prosopis (both of which are drought-resistant and well adapted to light, sandy soils).

Among many fast growing trees of potential commercial significance is the

Leucaena leucocephala (itself the subject of a separate book, Leucaena leucocephala: New Forage and Tree Crop for the Tropics, NAS, 1977). Resembling a mimosa, the tree can grow 65 feet tall and 16 inches thick in five years (roughly six times the rate of growth of an oak), giving wood suitable for paper pulp, furniture or building. Bushy varieties of the species help prevent soil erosion, while providing a nitrogenous fertilizer or a cattle feed rich in protein. Acacia auriculiformis, a robust and little known tree from New Guinea, can grow with exceptional vigour on problem soils such as eroding hillslopes, mining spoil, laterite and sand, as well as in highly acid and alkaline sites. More generally, the NAS report looks at other fast growing trees (especially of Acacia and Albizia species) and concludes that "many woody legumes are pioneer species that colonize newly cleared sites. To outdo the competition, they grow fast and are very precocious and vigorous trees that nodulate well. With the desperate need for reforestation, erosion control, firewood, and paper, as well as other wood products in developing countries, many such leguminous trees are worth widespread testing".

The rosewoods — classic furniture woods renowned for their gorgeous colours, beautiful grain and exceptional technical properties — come from species of *Dalbergia*, slowly growing leguminous trees found throughout the tropical regions of the world. Most species are being harvested to commercial extinction, and silvicultural research has been so neglected that in most cases it just is not known whether

the trees can be cultivated. The fascinating properties of several other slowly growing, 'luxury timber' trees are discussed in the NAS report. Here the problems of lack of information are compounded by the commercial reality that the 'discounted present value' of such slowly growing trees is relatively low, making their cultivation economically unattractive (see *Nature* 263; 91, 1976).

Last, but not least, legumes have given us many of the world's most exquisite flowering plants: wisteria, laburnam, poinciana, orchid trees, cock's comb coral tree, the raintree and many others. The NAS book lists and, better, illustrates the other beautiful leguminous species that should be far more widely known and more extensively planted, before they become extinct

# Small minded — the characterization of interplanetary dust by electron microscopy

from Colin Pillinger

In recent years, the extraterrestrial sample community has been made increasingly aware that a third category of material, besides meteorites and lunar rocks, is available for laboratory study. Samples of interplanetary dust may be collected in the Earth's stratosphere by appropriately equipped high flying aircraft. The grains, known as 'Brownlee particles' after Don Brownlee (of the University of Washington, and more lately the California Institute of Technology), who championed the cause of the collection programme, are now being collected in a programme coordinated by NASA from the Johnson Space Center.

Collecting cosmic dust is not a new pastime. Almost a hundred years ago the Challenger expedition recovered spherules of presumed cosmic origin from the slowly accumulating Pacific sediments1. Brownlee himself obtained copious quantities of extraterrestrial material from this source with his so-called 'cosmic muck rake', a magnetic device trailed behind a ship and capable of processing thousands of tonnes of sediment per day. Similar attempts have been made to find extraterrestrial debris in Arctic and Antarctic snow and a variety of other sediments. Perhaps the first report of micrometeorite debris can be attributed to an occurrence of 'Cryoconite' (Icedust) in Greenland ice2 reported in 1872.

Particle collection at high altitudes is possible because the braking effect of the terrestrial atmosphere is able to concentrate the incoming dust flux to around  $10^{-3}$  particles per m<sup>3</sup>. The frictional heating caused by deceleration depends on the size of the particle and its emmisivity. Very small grains (<10  $\mu$ m) do not reach anything like the melting point of the constituent material as they have been

slowed to terminal velocity from a speed of 25 km s<sup>-1</sup> at an altitude of 100 km (ref.3). Probably half the grains of size <10  $\mu$ m and with density  $\sim$  1 will not have been heated to greater than 550°C for more than two seconde<sup>4</sup>

That comparatively mild thermal history has meant that very flimsy low-density grains whose structures are often described as 'porous', 'reentrant' or even 'fairy castle' have survived for collection. That they are truly extraterrestrial is shown by rare gas contents and isotope abundance patterns which can only have been produced by exposure to the solar wind<sup>5,6</sup>. Solar flare particle tracks which might also provide evidence of space exposure have not, however, been found, suggesting that either some annealing took place during entry or the lifetime of interplanetary dust grains at 1 AU is much shorter than anticinated6

Availability of a special sample is one thing, participation in its study is another. Among the few who can sensibly contribute to the analysis of Brownlee particles is the group at the McDonnell Center for Space Sciences of Washington University, St Louis. Since 1976, this group, particularly P. Fraundorf, has been examining 11 particles in the size range  $4-15 \mu m$  by analytical techniques involving electron microscopy. Perhaps this five year effort and future studies on the same 11 grains are put in perspective by Fraundorf's analogy that a 5 µm grain to an electron microscopist is the same as a three ton hand specimen to a field geologist.

Fraundorf's efforts are now directed towards the chemical and mineralogical

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taxonomy of interplanetary dust particles and in a recent paper<sup>7</sup> he attempts to emphasize their differences, not only from known meteorite classes, but from each other. He has now become so familiar with his grains that each has acquired a name, some of which describe the characters of the specimens. Thus, 'Blowup' 'exploded' on crushing into hundreds of silicate fragments of wide-ranging composition each coated with some low atomic number material. 'Lost and Found' was predominantly a crystalline coarse-grained aggregate which was difficult to handle because of electrostatic charging.

Electron microprobe and single grain neutron activation studies have already shown8 that Brownlee particles have compositions which may be called 'chondritic', that is, similar to chondritic meteorites and relatively unfractionated compared with solar abundances. Fraundorf has looked more deeply into the compositional variations. One hundred and fifty analyses from regions as small as 10-15 cm3 from his 11 grains give average elemental abundance ratios which would be described as chondritic but on inter- and intra-grain scale the variations are extensive. In the case of meteorites, element abundance ratios can fairly uniquely define the class to which a sample belongs — none of the eleven grains could be assigned to a distinct chondritic group with any certainty.

Plotted on the standard Urey-Craig plot (reduced iron + sulphide/Si versus oxidized iron/Si), interplanetary dust particles (IDPs) fall in the region ascribed to the most reduced H, L and E chrondrites, samples which often show some degree of metamorphism. None of Fraundorf's eleven grains plots with the oxidized carbonaceous chondrites,

samples generally thought to be the most primitive (least metamorphosed) meteorites, even though numerous arguments exist to show that IDPs are relatively unmetamorphosed and probably contain per cent quantities of carbonaceous phases (low atomic number material).

It would be easy to see how incoming IDPs were oxidized during entry but not reduced; the overall oxidation state of IDPs must reflect their formation in a highly reduced region of the primitive solar nebula. Fraundorf points out from his mineralogical studies that some of the finegrained magnetite decoration on crystal surfaces might be due to atmospheric heating, but could just as easily fit characteristics predicted by core/mantle models for interstellar grains.

Before Fraundorf's studies, only X-ray powder diffraction had been used to recognize minerals in whole IDPs (ref.9). Now as a result of both single- and polycrystal electron diffraction studies, coupled with dark and bright field imaging and consideration of concurrent chemical analysis data, magnetite, pyroxene, olivine, pyrohotite, taenite and cohenite (the latter as a possible deposition product from a reducing gas phase) have all been identified. Perhaps just as important is that amorphous zones of a particular chemistry (such as S/Fe > 2) have been observed. One surprise is the absence of hydrated silicates, again distinguishing IDPs from the matrix material in carbonaceous chondrites. The possibility that water was lost by exposure in space or atmospheric entry cannot be discounted but the evidence seems more and more that Brownlee particles are distinct from known meteorite classes and really are a third category of extraterrestrial sample.

It is hoped that soon many other investigators will become small minded and accept the challenge of Brownlee particles. The full characterization of primitive materials and their taxonomic classification is a necessity to our understanding of the early Solar System. It is still largely supposition that IDPs are derived from cometary sources but the indirect evidence to this origin continues to grow. Just the conjectured availability of cometary dust alone should mean a tremendous crossfertilization between the laboratory-bound analysts and the spacecraft experimenters who hope to receive data from the ESA Giotto mission to Halley in 1986.

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# Clues for the origin of cosmic rays

from Peter Meyer

DURING the 1960s and early 1970s it became possible to measure the elemental composition of the cosmic radiation in fine detail. This achievement constituted a major breakthrough towards recognizing the elusive origin of the high energy particles that pervade the Galaxy. In particular, it was found that elements over the entire range of the periodic system are present in the cosmic radiation. that the relative abundance ratios of the cosmic ray elements are strikingly similar to those found in the elements that make up the Solar System. Hoyle first made the suggestion, now generally accepted, that the chemical elements heavier than lithium are produced by nucleosynthesis in the interiors of stars. It seems clear that the cosmic ray particles cannot be primordial, but rather must have been part of stellar material before their acceleration.

The crucial questions remaining to be answered concern the identification of the sites of origin and of the acceleration mechanisms responsible for the high energy particles. From the point of view of energy alone, it is clear that a powerful particle accelerator must be available. The cosmic ray population must be replenished, on the average, every ten million years, which is the currently accepted value of the mean storage time of the particles in the Galaxy before escape. This population is known to have existed with an intensity similar to that now observed throughout the lifetime of the Solar System, that is, about 4,500 million years. Supernova explosions are the most energetic known phenomenon in the galaxy, and were therefore long suspected to be responsible for the origin of cosmic ray and possibly to imprint characteristic

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abundance distributions on the ejected

Major theoretical work has led to a deeper understanding of various nucleosynthesis processes and in attempts to describe the elemental and isotopic composition of the Solar System, the end products from hydrostatic and explosive nucleosynthesis were investigated. Explosive syntheses are expected to occur on a short time scale in conditions similar to those observed in the supernova phenomenon. Hence, the deviations of cosmic ray elemental and isotopic compositions from the Solar System or interstellar matter abundance distributions provide information that reveals the origin of the particles.

Two recent publications Astrophysical Journal Letters (1 August 1981)address this question in two different ways. One paper deals with the isotopic abundance of several elements of low atomic number, the other with the elemental abundances of nuclei with atomic number between 26 and 40 (Fe to Zr)2. The abundance distribution of the elements lying between helium and nickel in atomic number is not a particularly sensitive discriminator between the nucleosynthesis processes forming the cosmic ray elements, but the isotopic composition is. The discovery of an 'anomaly', that is, a deviation from Solar System abundance, in the isotopic composition of the element neon was therefore received with great excitement3-8.

The most accurate results were obtained from two high-resolution experiments carried out on the spacecraft ISEE-3, for the Caltech group<sup>5</sup> and for the Berkeley group<sup>1,8</sup>. They present additional, new values for the abundance ratios <sup>22</sup>Ne/<sup>20</sup>Ne,  $^{21}$ Ne/ $^{20}$ Ne,  $^{25}$ Mg/ $^{24}$ Mg,  $^{26}$ Mg/ $^{24}$ Mg, <sup>29</sup>Si/<sup>28</sup>Si and <sup>30</sup>Si/<sup>28</sup>Si. It is claimed that all these isotopic ratios show enhancements of the neutron-rich isotopes over Solar



# 100 YEARS AGO

THE conduct of competitive examinations in China seems to be farther from perfection than might be expected in the case of such an ancient institution. The Peking Gazette contains a memorial from one of the censors complaining that the matsheds which are erected at the entrance to the examination hall in the capital to issue tickets of admission to competitors are frequently overturned by the rush of applicants, that an unseemly crowding and snatching of tickets from the officials take place, and that candidates break the rule prohibiting them from leaving the compartments in which they are isolated during the examination. They are allowed, he says, to fetch their food themselves (examinations in China last from thirty-six hours to three days at a stretch) from the kitchens, and they meet and converse freely. Prepared essays, the memorialist fears, are passed in from outside during these hours by the student's friends. Again, when the lists of successful candidates are posted up, a tumultuous crowd assembles outside the gates; bands of the unsuccessful ones obstruct the progress of the chief examiner, employing threats and entreaties to prevail on him to alter the lists.

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System abundances. This enhancement amounts to a factor of about 1.6 except for the ratio of 4.1 measured for <sup>22</sup>Ne/<sup>20</sup>Ne. It is not yet understood why the Ne isotopes show a larger anomaly; a theoretical model by Arnett<sup>9</sup>, extended to the special case of these cosmic ray isotopes by Woosley and Weaver<sup>10</sup>, would predict all the ratios to be of the same order.

There is no doubt that the isotopic ratios for the above cosmic ray nuclides differ from the standard Solar System values. The critical reviewer of this work remains concerned that the standard Solar System values are always quoted without errors, which are obviously difficult to estimate. A most interesting comparison is made by Wiedenbeck and Greiner with recent spectroscopic determinations of the silicon isotopes in interstellar clouds<sup>11</sup>. In some interstellar clouds an enhancement of the neutron-rich isotopes 29Si and 30Si is observed which is not dissimilar to that seen in the galactic cosmic rays. If such observations turn out to be representative for the interstellar medium, the anomalous isotopic composition of the cosmic rays remains compatible with their source being pre-existing interstellar material which itself carries those anomalies.

The second sensitive indicator for the nature of the cosmic ray sources is the chemical abundance distribution of the very rare ultraheavy elements with atomic number byond that of the iron/nickel group. Attemps to measure that abundance distribution date back to the late 1960s, only a few years after the discovery of ultraheavy elements in the cosmic radiation. The early measurements pointed towards an enhancement of elements produced by rapid nucleosynthesis. The work by both Binns et al.2, carried out on the spacecraft HEAO-3, and Fowler et al. 16, on ARIEL-6, has much improved charge resolution. Binns et al. conclude that, within the charge range of Z from 26 to 40, the HEAO-3 results are inconsistent with a cosmic ray source that is dominated by r-process nucleosynthesis, but quite

consistent with an elemental abundance distribution similar to that of Solar System material. A crucial element ratio supporting this conclusion is the Se/Sr abundance ratio, found to be about ten times that expected from the r-process.

Clearly, the recent work on isotopic anomalies as well as on the abundance distribution of superheavy elements in the cosmic rays is consistent with the interpretation that the source of the particles may be the interstellar medium. It is important to note that these papers represent interim results, that are expected to be improved upon, and that in both directions of research much remains to be done. Several crucial isotopic ratios  $(^{34}S/^{32}S, ^{54}Fe/^{56}Fe, ^{58}Fe/^{56}Fe)$  must be

determined accurately with instruments having higher sensitivity than exists at present; this work should go hand in hand with new attempts to determine the corresponding isotopic abundances in the interstellar medium. The potentially very revealing elemental distribution of elements with Z > 40 must be measured accurately. The Pt/Pb ratio and abundance of the actinides are particularly crucial in identifying the origin of the particles. Some time in the future measurements of isotopic composition may include the ultraheavies; this would provide a powerful tool not only for determining the sources of cosmic rays, but also for understanding the contemporary interstellar medium.

# Middle atmosphere dynamics

from John Gregory

MUCH of our understanding of the dynamics of the middle atmosphere 1-9 comes from data gathered in the mid-sixties by rocket techniques and by meteor radars. The techniques each had their particular limitations and left the mesosphere and lower thermosphere (60-100 km above the Earth's surface) as the least known regions of the atmosphere. In the 1972 Cospar International Reference Atmosphere2, for example, data were insufficient to assemble a latitude-season cross-section of meridional winds, 60-120 km above the Earth's surface, while the zonal crosssection was incomplete for the higher latitudes and altitudes. This situation provided the impetus to set up a coordinated program of international scientific research, the Middle Atmosphere Program (MAP).

In the last decade, two new techniques have provided additional data. First, satellite-borne radiometers 10,11 have improved global coverage of the stratosphere and lower mesosphere, to 80 km, notably with respect to the large-scale temperature structure and its perturbation by planetary waves and stratospheric warmings. Weighting factors broaden with altitude and result in decreasing altitude discrimination and this, coupled with low temporal resolution, means that data for the study of motions such as tides and gravity waves may not be readily available from these techniques. The derivation of geostrophic winds from temperatures, after inversion of radiances, requires knowledge of pressure fields at a lower boundary, such as is available from balloon and rocket sondes.

The second technique comprises ground-based radar, in several variants, to which may be allied lidar. The oldest form of radar measurement of winds12, in which

a moving diffraction pattern at ground level is sampled at spaced antennae, has been applied to MF partial radiowave reflections<sup>13-15</sup> from irregularities in electron density. Examples of the wind field determined by fully automated version of this method are shown in Figs 1 and 2. A later form16-20, in which Doppler measurements of winds are made at VHF, utilizes reflections from electrons in the mesosphere, and aerosols in the troposphere and lower stratosphere. In the region 30-60 km, irregularities in neutral density provide some scattering, but extremely sensitive radars are required to detect it. These two variants of radar, once conceived as differing in principle, have been shown<sup>21</sup> to be based on the same scattering irregularities. Thomson scattering from electrons at UHF can be processed to yield winds, 60-100 km<sup>22</sup>. Lidars<sup>23,24</sup> are now being employed to derive winds and density profiles, 30-80 km. The altitude resolution of the radar techniques is from 0.2 to 3 km, and is thus adequate for studies of gravity waves and tides. VHF and UHF radars are advantageous for turbulence studies.

Available techniques are already providing data for MAP projects in dynamics, in particular, a coordinated study of the behaviour of the middle atmosphere in winter (MP-1; Chairman, K. Labitzke) and equatorial wave dynamics (MP-2; Chairman, I. Hirota). Study groups have identified observational requirements for dynamical problems for example, the role of tides, gravity waves and turbulence in respect to momentum and energy budgets (Study Group 3;

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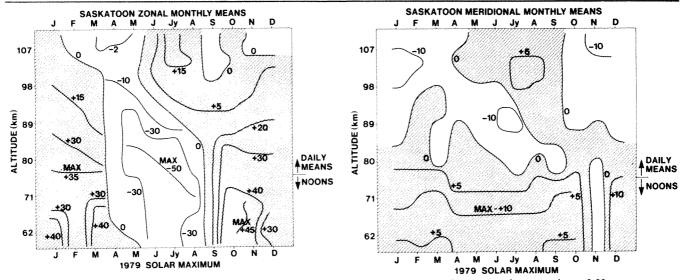
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The wind field in the mesosphere and lower thermosphere, as determined by spaced-antenna radar, operating at 2.22 MHz, in a fully-automated mode, at Saskatoon, 52N, 107W. The zonal flow, Fig. 1, is in good agreement with European results at similar latitudes, but the meridional flow, Fig. 2, shows a poleward component below 80 km in summer, contrary to model calculations.

Chairman, M.A. Geller), also the role of gravity waves and turbulence in respect to transport of minor constituents (Study Group 2; chairman J.D. Mahlman).

Techniques are potentially available for the majority of probable programmes, with two major exceptions. Gravity waves and turbulence are at present amenable to study only by ground-based and rocket techniques so the determination of their global characteristics seems a goal that is unlikely to be met. Also, measurements of mean vertical speeds, which range from cm per second in the troposphere to m per second in the lower thermosphere, are not yet practicable. The two related techniques, Doppler and spaced-antenna radar, are in principle capable of doing so, but there are economic as well as technical limitations. Finally, it may be noted that satellite measurements of winds by Doppler optical and microwave techniques are under development, but mainly due to spacecraft scheduling, are unlikely to produce data for about a decade.

Models of global circulation have been developed from basic equations of motion and knowledge of radiation fluxes and constituent concentrations3-9. A second approach has been to calculate the meridional circulation, including vertical motion, from observed radiance fields25. So far, only zonal mean motion has been modelled. Most models are able to reproduce the major features of zonal mean flow in the stratosphere and mesosphere, for example, the winter eastward and summer westward flow (see Fig. 1). A major difficulty which seems to have been encountered in nearly all models is the development of excessive Coriolis torques from meridional flow, the latter being derived from the requirement that vertical motion compensate for observed departure from radiative equilibrium, for example, the cold mesopause in summer. In consequence, computed zonal speeds are generally much in excess of those observed, and modellers have resorted to imposing arbitrary damping. Since planetary waves have now been excluded from the list of possible sinks of momentum in the summer mesosphere<sup>26</sup>, opinion currently favours tides and gravity waves as the damping mechanisms. It appears that before significant advances in modelling mean flow can be made, observational and theoretical studies of tides and gravity waves, together with their interaction with mean flow, must progress.

Much of the theoretical study and modelling of specific dynamical features, for example, the quasi-biennial and stratospheric oscillation<sup>27</sup> warmings<sup>28,29</sup>, as well as the global circulation, preceded MAP, and would undoubtedly have proceeded independently of it. The purpose of MAP is to facilitate such work, and this is possible in two ways. First, desirable observational programs are indentified. The space and time scales appropriate to each dynamical process are determined. Thus, for example, attention has been drawn to the relative lack of observations in the tropical upper mesosphere. Member nations of The Inter-Union Special Committee on Solar Terrestrial Physics (SCOSTEP) are encouraged to determine what contribution they can make to global observational programmes, or what work they may specifically attempt in their territory. Thus European nations have made special studies of winter middle atmosphere conditions, and are planning more.

Second, the systematic compilation of data is to be attempted. This is intended to permit the assembly of a dynamic climatology of the middle atmosphere, initially between 30 and 60 km, and as data become available, between 60 and 120 km. In addition, the data are intended to form a source for incorporation into models, and a reference for comparison. In particular, such data will, it is hoped, serve to guide aeronomers concerning the transport of trace constituents, in respect to which an 'eddy diffusion coefficient' is currently utilized. It is well appreciated that many of the aeronomic problems of the middle atmosphere, for example. concentration of water vapour in the summer mesosphere, or of electrons in the winter mesosphere cannot be determined by photochemical studies alone, and a major aim of MAP is to ally these latter to appropriate dynamical studies.

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# **REVIEW ARTICLE**

# The early Universe

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In the past few years one of the most exciting areas of research in physics has been the interdisciplinary field of cosmology and particle physics. The NSF's Institute for Theoretical Physics in Santa Barbara devoted a 6-month program and an intensive 1-week workshop to the subject. A brief review is given of both the workshop and this field which is attracting attention, in part, because the early Universe seems to be the only laboratory in which to study grand unification.

AT the Institute for Theoretical Physics (ITP) on the campus of the University of California at Santa Barbara, the NSF is trying a new approach for tackling problems in theoretical physics. The idea is to bring together for 6-months to 1-year periods people from all over the world who are working on similar problems, in the hope that their collective effort will be more productive than the sum of what the individuals could accomplish working separately. Usually several different workshops run concurrently, and so there is the possibility that connections between apparently disconnected areas of physics will be found.

From January to June 1981, a program was held on the early Universe, which focused on the close relationship between cosmology and particle physics. Simultaneously, there were related programs in the areas of quantum gravity and gauge field theories. Within the past 5 years there has been much exciting work and interesting developments in the interdisciplinary field of particle physics and cosmology. The primary tool of cosmologists has been the large telescope, but now they are interested in deep underground mine experiments built to detect proton decay, in experiments on mountain tops searching for rare cosmic ray events, in experiments performed at accelerators and reactors which are designed to detect axions and neutrino oscillations, in balloon flights looking for antiprotons, in high altitude U-2 flights which carry instruments to measure anisotropies in the 3 K microwave background, in low temperature searches for fractional charge states, in deep underwater muon and neutrino detectors, and even in a proposed monopole search at an iron-ore processing plant.

Traditionally, the large accelerator has been the primary tool of the particle physicist, however, they are now interested in reactor experiments, in deep underground mine experiments, in bulk matter searches for exotic particles left over from the big bang, in the cosmic abundance of helium and deuterium, in the distribution of galaxies, in the structure of the microwave background, and even in experiments designed to detect solar neutrinos. It is not uncommon to find as many cosmologists as particle physicists at a particle physics seminar and vice versa. The connection between the two disciplines is, of course, the big bang. In the early Universe temperatures as high as  $10^{32}$  K were reached, corresponding to average particle energies as high as 1019 GeV. The particles present and their interactions determined the early evolution of the Universe, and the type of relics that remain. Particle physicists are constantly seeking higher energies where the world appears simpler, and the early Universe seems to be the only laboratory with large enough energies (≥10<sup>14</sup> GeV) to study models of the unification of the strong, weak and electromagnetic forces. Cosmologists are asking particle physicists if neutrinos have small rest masses, and if the proton is unstable. If neutrinos have small rest masses they may determine the large-scale dynamics of the Universe; and if the proton is unstable, baryon number violating reactions may

explain why the Universe is predominantly matter, rather than having equal amounts of matter and antimatter. Conversely, particle physicists are asking cosmologists about the cosmic environmental impact of exotic particles which either cannot be produced at accelerators or would have escaped detection.

The culmination of the early Universe program at Santa Barbara was a week-long workshop which brought together experimental and theoretical high energy physicists, theoretical astrophysicists, astronomers and cosmologists. The workshop focused on many of the recent exciting developments and the important outstanding problems of common interest in both particle physics and cosmology. We shall now highlight some of the work presented at the meeting, and review the present status of the field of cosmology and particle physics.

# Standard big-bang model

It is now generally accepted, and supported by observations that the Universe began from a hot big bang (for review see refs 1-3). The evidence includes: the universal expansion or 'Hubble flow'; the 3K cosmic microwave background; the large abundance of D and  ${}^4\text{He}$ ; and on the theoretical side, the singularity theorems of Hawking and Penrose. In the homogeneous and isotropic Friedmann-Robertson-Walker (FRW) model, the scale factor of the Universe, R(t), is governed by

$$(\dot{R}/R)^2 = H^2 = 8\pi G\rho/3 + \Lambda/3 - k/R^2 \tag{1}$$

where  $\rho$  is the total energy density,  $\Lambda$  is a possible cosmological term, and k is the curvature signature, which when R(t) is appropriately scaled is 0 (open and flat), 1 (closed and positively curved), or -1 (open and negatively curved). The energy density of radiation  $\propto T^4 \propto R^{-4}$  as  $T \propto R^{-1}$ , and the energy density of matter  $\propto R^{-3}$ . For  $T \ge 10^5$  ( $\Omega h^2$ )K ( $t \le 3 \times 10^{10}$  s  $\Omega^{-2}$   $h^{-4}$ ), radiation dominates the energy density and the right-had side of equation (1), as it increases faster than the other two terms as  $R \to 0$  ( $\Omega$  is the present ratio of the energy density to the critical energy density, and the Hubble parameter H = 100h km s<sup>-1</sup> Mpc<sup>-1</sup>). During the radiation epoch, the age and temperature of the Universe are related by

$$t = \left(\frac{45}{16\pi^3 G}\right)^{1/2} g_*^{-1/2} T^{-2} = 2.41 \text{ } \mu \text{s } g_*^{-1/2} T_{\text{GeV}}^{-2}$$
 (2)

where  $g_*$  is the total number of degrees of freedom of all species which are ultrarelativistic (that is species with  $m \ll T$ ), and throughout units are used such that  $\hbar = c = k_{\rm B} = 1$ . For  $t \ge t_{\rm Planck} \sim 10^{-43}$  s, quantum corrections to general relativity should be small, and equations (1) and (2) should be valid. When T is much greater than the mass of a particle, that particle and its antiparticle are about as abundant as the photons. So for  $T \ge 10~{\rm GeV}$  ( $t \le 10^{-8}$  s), all the particles which we have produced at accelerators ( $e^\pm$ ,  $\mu^\pm$ ,  $\tau^\pm$ ,  $\nu\bar{\nu}$ , and all the quarks) were as abundant

as photons. Earlier than  $t \sim 10^{-12}$  s, average particle energies were greater than 1,000 GeV—the highest energies achieved at particle accelerators. At  $t = t_{\rm Planck}$ ,  $T \sim 10^{19}$  GeV. It is clear why the early Universe has been called the ultimate particle accelerator.

# Elementary particles and interactions

In the past decade or so much progress has been made in the understanding of fundamental particles and their interactions: the strong, weak, and electromagnetic forces (for review see ref. 4). There is now good evidence that the fundamental particles of nature are quarks-five different types are known to exist, and a sixth type is also believed to exist (u, d, c, s, b and t), and leptons—six different types are known (e<sup>-</sup>,  $\nu_e$ ,  $\mu^-$ ,  $\nu_\mu$ ,  $\tau^-$  and  $\nu_\tau$ ). Quarks and leptons apparently come in pairs: (u, d) and ( $\nu_e$ , e<sup>-</sup>); (c, s) and  $(\nu_{\mu}, \mu^{-})$ ; (t, b) and  $(\nu_{\tau}, \tau^{-})$ . Each quark also comes in three colours. It is now believed that each of the fundamental forces can be described by a gauge theory, in which the interactions are mediated by gauge bosons. Each gauge theory is distinguished by an invariance group, usually unitary (symbol U(n)) or special unitary (symbol SU(n)), whose elements are 'rotations' on an n-dimensional complex vector. Each gauge theory has its particular group (and value of n) which distinguishes it from all others: it has particular algebraic relations among its charges, and among the gauge fields that emanate from them, which are determined by the group—which thus determines the physical nature of the fields described.

For example, the gauge theory of electromagnetism is a U(1)theory, and the gauge field is the electromagnetic field whose particle representation is the photon. The colour force between quarks is described by an SU(3) gauge theory. The gauge fields are the colour fields, and their particle representations are called gluons. The strong forces between nucleons and other baryons and mesons are believed to be a residual effect of the colour force, analogous to van der Waals' forces. The weak and electromagnetic interactions can be described by one unified theory, the  $SU(2) \times U(1)$  gauge theory. (Here each element of the direct product gauge group is composed by taking the product of one element from U(1) and another from SU(2).) In this theory the gauge particles are the photon,  $W^{\pm}$  and Z bosons. Normally these bosons, like all primitive gauge bosons, would be massless and long range, but in the Weinberg-Salam model which uses the  $SU(2) \times U(1)$  gauge group an additional concept is introduced: that the vacuum is not empty but uniformly filled with certain scalar fields, the Higgs fields, which possess a subset of the charges of the gauge group and slow the propagation of-or give mass to-the corresponding subset of gauge bosons. Here these are taken to be W<sup>±</sup> and Z' are given masses of around 80 GeV. This process called 'spontaneous symmetry breaking' has a characteristic energy scale below which the gauge symmetry is broken (and the relevant gauge bosons get mass) and above which the full symmetry is restored (and all the gauge bosons are massless). (The usual analogy used for spontaneous symmetry breaking is ferromagnetism. Maxwell's equations are rotationally invariant; however, below the Curie temperature the rotational invariance of a ferromagnet is spontaneously broken when the magnetization chooses a specific direction.)

Encouraged by the success of the  $SU(2) \times U(1)$  unified gauge theory, particle theorists have speculated that all three forces are unified under one gauge group (not yet determined) which is spontaneously broken. In the simplest class of models, the breaking scale has been calculated to be  $\sim 10^{14}$  GeV (ref. 5). The unification of the three interactions requires new interactions, interactions which violate baryon and lepton number conservation. These two conservation laws were never very pleasing; unlike charge or energy and momentum conservation, these quantities do not couple to long-range forces.

The startling prediction of grand unified theories (GUTs) is the instability of the proton, with a lifetime of  $0(10^{30} \text{ yr})$ . At least five large underground experiments designed to detect proton decay are under construction, and several smaller experiments

are operating (for review see ref. 6). A Japanese-Indian collaboration in the Kolar Gold Fields has recently reported three candidate events for proton decay, translating into a proton lifetime of  $\sim 3 \times 10^{30}$  yr (ref. 7).

The simplest picture of unification predicts no new interactions between 300 GeV and  $10^{14}$  GeV; however, there very well may be new interactions yet to be discovered. There is, of course, the problem of also unifying gravity—'superunification'. Grand unification seems to occur ~4 orders of magnitude lower than the scale at which gravity should be as strong as the other forces (~ $10^{19}$  GeV), and it is hoped that gravity can be unified independently, at a later stage. At present, various efforts are being made to obtain a quantum theory of gravity, and even unify gravity (for example, supergravity theories).

In the gauge theory description the world becomes simpler at higher energies-full symmetry is restored, and the forces are predicted to become weaker, so that the Universe, during its earliest stages when particle number densities were as high as 1099 cm<sup>-3</sup>, can be treated as an ideal gas of weakly interacting point-like particles. Clearly the early Universe is the only laboratory in which the ultrahigh energies that particle physicists are now interested in were achieved. Unfortunately, this laboratory shut down over 1,000 Myr ago, so that one must search for fossils which remain from the earliest epochs. The fossil record to date includes: the existence of structure in the Universe (galaxies, clusters, and so on), the baryon-to-photon ratio  $\approx (3-5) \times 10^{-10}$ , the abundance of D and <sup>4</sup>He, and the 3K microwave background and its angular structure, the absence of a large cosmological term or curvature term (that is, both are less than, or of the order of the matter density term), the possible existence of fractionally charged states, and the absence of various exotic species that might have been present if various proposed particle physics theories were correct.

# Primordial nucleosynthesis

One of the great triumphs of the hot big-bang model is primordial nucleosynthesis (for review see ref. 8). During the period  $\sim 1-200$  s after the 'bang' when T was  $\sim 1-0.1$  MeV,  $\sim 25\%$  of the mass of the Universe was synthesized into <sup>4</sup>He, with lesser amounts of D, <sup>3</sup>He and <sup>7</sup>Li being produced. The nucleosynthetic yields depend on nuclear reaction rates which are measured in the lab,  $\eta$  (the ratio of nucleons to photons), and the expansion rate of the Universe which itself is determined by  $g_*$ , the number of effectively relativistic species present [see equation (2)]. By using the observed abundance of <sup>4</sup>He, which is the sum of the primordial and stellar produced <sup>4</sup>He, big-bang nucleosynthesis has been used as a very powerful probe of this early epoch. Specifically, one can set an upper limit to the number of light (<1 MeV) neutrino species, as they would have been present and contributed to  $g_*$  during this epoch. However, to do this, one needs a lower bound to  $\eta$ . If one assumes that baryons (and not, for example, neutrinos) dominate the mass of small groups of galaxies, then dynamical studies of these systems provide such a bound, and the limit of ≤4 neutriono species has been obtained. In quark-lepton symmetric theories this implies ≤8 quark flavours9

At the Santa Barbara workshop Steigman reported new work 10 which uses the D and 3He abundance to obtain a lower bound to  $\eta$ , and also results in the limit of  $\leq$ 4 neutrino species, independent of whether or not baryons dominate the mass of small groups of galaxies. Note that this lower bound is consistent with that obtained from studies of small groups of galaxies, and so is evidence that small groups are dominated by baryons. Studies of rich clusters of galaxies result in a much higher lower bound to  $\eta$ , indicating that they are not predominantly baryons, but perhaps neutrinos. In addition, when 4He is used to obtain an upper limit to  $\eta$ ,  $\eta$  is restricted to the range  $3-5\times10^{-10}$ , or  $\Omega_{\rm B} \cong (0.01-0.02)\ h^{-2}(T_{\gamma}/2.7\ {\rm K})^3$ . Thus, nucleons alone cannot close the Universe. With this new lower bound, the standard model makes the firm prediction that the primordial 4He production is 0.23-0.25. The primordial mass fraction of 4He must be less than the presently observed fraction of 4He. 4He

observations range from 0.23 to 0.32 with some of the values having stated errors as small as  $\pm 0.01$  or 0.02. The higher values can be accounted for by stellar production (and indeed usually involve systems with normal metal abundances, indicating that stellar processing has occurred). If the primordial mass fraction of <sup>4</sup>He is found to be less than 0.23, then the standard model is in trouble <sup>11</sup>.

If this should occur there are several alternatives: a cold big bang in which the 3 K background must be produced astrophysically  $^{12.13}$ ; perhaps some of the primordial D and  $^3$ He (or  $^4$ He) was destroyed by astrophysical processes; or a large lepton number might be hidden in the undetectable neutrino seas. In the standard model of primordial nucleosynthesis it is assumed that the lepton number-to-photon ratio, like  $\eta$ , is  $\ll 1$ . Reeves discussed primordial nucleosynthesis when the lepton number-to-photon ratio is of 0(1) (ref. 14). Relaxing the assumption that the lepton number is small allows much more freedom, and it is possible to explain simultaneously a low primordial  $^4$ He abundance, and the observed abundances of D and  $^3$ He. Although baryosynthesis usually results in lepton and baryon numbers of the same order of magnitude, one of us (E.W.K.) discussed a GUTs scenario which results in  $\eta \sim 10^{-10}$  and a large lepton number  $^{13}$ .

#### Baryosynthesis

Although the laws of physics are almost exactly matter-antimatter symmetrical at the microscopic level (the only exception is a small effect in the neutral kaon system), the Universe appears to be grossly asymmetrical. There is no evidence for the existence of appreciable amounts of antimatter in our Galaxy. If antimatter exists in the Universe in non-negligible amounts, then the size of matter or antimatter domains must be greater than the size of clusters of galaxies<sup>16</sup>. The matter asymmetry is usually quantified as the baryon number, or more exactly the baryon-to-photon ratio  $B = (n_b - n_5)/n_{\gamma} \cong \eta$ , where  $n_b$ ,  $n_5$ , and n, are, respectively, the number densities of baryons, antibaryons and photons. (For review of baryosynthesis, see refs 17, 18.) The Universe today has  $B = 3-5 \times 10^{-10}$ ; for  $t \le 10^{-6}$  s after the 'bang' ( $T \ge 1$  GeV), baryons and antibaryons were as abundant as photons, and this ratio then translated into a matter-antimatter fractional asymmetry of ~1 part in 1010. Perhaps the most exciting application of GUTs to cosmology is the possible explanation of the baryon asymmetry. Sakharov<sup>19</sup> discussed the conditions necessary for an initially symmetrical universe,  $n_b = n_b$ , to evolve into an asymmetrical universe,  $n_b \neq n_{\bar{b}}$ . The three ingredients outlined by Sakharov are: baryon number nonconserving interactions; C- and CP-violating interactions, that is, interactions which violate matter-antimatter symmetry; and a departure from thermal equilibrium. The necessity of baryon number violation and interactions that distinguish between particles and antiparticles (CP-violation) is clear. As the entropy of a system is a maximum when the baryon number vanishes, a departure from thermal equilibrium is also necessary for the microscopic interactions to produce the asymmetry.

In GUTs quarks and leptons are usually grouped together in a unified manner, and so there is a gauge equivalence between leptons and quarks. Physically, this gauge equivalence corresponds to interactions mediated by gauge bosons (which, of course, violate baryon number conservation). As C- and CP-violations are observed in the kaon system, it is not unreasonable to expect such violations to occur also in baryon number violating reactions. The expansion of the Universe can provide the final condition: if the expansion is rapid enough, then the temperature of the universe  $(T \sim R^{-1})$  changes too fast for particle interactions to maintain equilibrium. All three of these ingredients are incorporated into the out-of-equilibrium, drift and decay (British) model proposed by Weinberg and Wilczek<sup>20,21</sup>.

The generation of the baryon asymmetry has motivated many people to take the idea of unification seriously enough to study various grand unified models in detail. In fact, until proton decay is seen, the existence of a cosmological baryon asymmetry is the only "experimental" evidence for baryon number violation. Several detailed calculations of the generation of the baryon number were presented at the conference.

Although the standard scenario for the development of the baryon asymmetry has rapidly gained wide acceptance, there are still basic uncertainties in the model. One uncertainty involves the assumption of thermal equilibrium as an initial condition. The effect of non-equilibrium initial conditions was discussed by one of us (M.S.T.)<sup>22</sup> who showed that the magnitude of the asymmetry produced can be different if the Universe was not intially in a state of thermal equilibrium. Another uncertainty is the magnitude and type of CP-violation. It seems almost certain that the baryon asymmetry cannot be directly related to the CP-violation in the K°-K° system. Nanopoulos23 presented a model that related the CP-violation responsible for the baryon asymmetry to the neutron electric dipole moment, and not to the kaon system. (The electric dipole moment of the neutron is another possible manifestation of CP-violation.) The calculations suggest that the neutron electric dipole moment should be just smaller than the present upper bound.

Although many details need to be worked out, there is a feeling that a solution to the fundamental cosmological problem of the origin of the baryon asymmetry is at hand. A possible problem is the detection of low-energy cosmic ray antiprotons by Buffington et al.24 discussed at the conference by Gaisser. Although the antiprotons (p) detected are small in number (≈14) and small in proportion to the protons detected (p̄/p≈ 10<sup>-4</sup>), and although ps are expected to be produced as secondaries in high-energy cosmic ray collisions, the energy of the antiprotons detected are too low to be accounted for by standard galactic cosmic ray models. Several possible explanations were discussed at the workship: (1) The experiment is wrong. (2) The galactic models are grossly in error. (3) The ps were originally produced at high energies and then decelerated without annihilating. (4) Cosmic ray neutrons oscillate into antineutrons which then decay into antiprotons. (5) The antiprotons are primary cosmic rays originating from antigalaxies 23 . Although there was no consensus on the interpretation of the experiment, there was agreement that if the experiment is correct, the implications will be very significant.

#### Galaxy formation

On the largest scales (>10 Mpc) the Universe is quite smooth and homogeneous, while on small scales (\$10 Mpc) it is lumpy (nuclei, atoms, molecules, creatures, planets, stars, galaxies, clusters and superclusters). The large-scale smoothness, the uniformity of the microwave background, and the absence of a profusion of black holes are evidence that as early as recombination  $(T \sim 4,000 \text{ K}, t \sim 10^6 \text{ yr})$  the Universe could be described by an FRW model supplemented by small density perturbations. The concordance of the primordial nucleosynthesis calculations with the observed abundances of D and <sup>4</sup>He, probably indicates that this description was valid as early as  $\sim 1$  s after the 'bang'  $(T \sim 10^{10} \text{ K})$ . It is generally believed that small density fluctuations, present at recombination, after being freed from the pressure support provided by the radiation, collapsed due to their self-gravitational attraction and evolved into the structure we observe today. This is where the general consensus ends. The precise details of the growth of the density perturbations since recombination, their initial spectrum and origin are all areas of intense research and debate (for a review of galaxy formation, see ref. 26).

During the radiation dominated epoch  $(T > 10^5 \, \mathrm{K} \, \Omega h^2)$  density perturbations can be divided into two classes: isothermal and adiabatic. The subsequent development of structure is very different for these two types of initial fluctuations. Isothermal perturbations are characterized by uniform radiation temperature, with spatial variations in the beryon number-to-photon ratio. After recombination isothermal perturbations grow on all scales  $> 10^5 \, M_\odot \, (M_\odot = 1 \, \mathrm{solar \ mass} \simeq 2 \times 10^{33} \, \mathrm{g})$ . Structure forms on the smallest scales first, which then themselves cluster,

and so on in a hierarchical manner. Adiabatic fluctuations are characterized by spatial variations in the temperature, but constant baryon number-to-photon ratio. During recombination, fluctuations on scales  $\leq 10^{14} \, M_{\odot}$  are damped by photon diffusion, so that the first structures to form are  $10^{14} \, M_{\odot}$  pancake-like objects (which are now identified with superclusters), which subsequently hydrodynamically fragment into galaxies, and so on.

Two recent developments in particle physics and cosmology have had important implications for theories of galaxy formation: baryosynthesis and the possibility of non-zero neutrino masses in the range 10-100 eV. Shortly after the idea of baryosynthesis was first discussed it was shown that if the Universe was nearly FRW during the epoch of baryosynthesis, it was not possible to produce a baryon asymmetry and isothermal fluctuations simultaneously—thus favouring the adiabatic picture<sup>27</sup>. However, at the Santa Barbara workshop two groups reported that in a universe initially dominated by large-scale inhomogeneous shear, isothermal perturbations can be produced during baryosynthesis<sup>28,29</sup>.

The adiabatic picture predicts temperature fluctuations of  $0(10^{-3})$  in the microwave background on small angular scales  $(\theta \ge 10'')$ , which do not agree with the observations. Massive neutrinos may save the adiabatic hypothesis. Last year a Russian group reported evidence that the electron neutrino has a mass  $0(30 \, \text{eV})$  (ref. 30) (GUTs can very naturally accommodate such neutrino masses). A neutrino of mass  $\sim 3 \, \text{eV}$  would dominate the mass density of the Universe; a neutrino of mass  $\sim 100 \, \text{eV}$  would provide closure density. Bond and Szalay reported that when massive neutrinos are added to the adiabatic picture, smaller initial perturbations are needed, resulting in predicted microwave fluctuations which are smaller than the present observational upper limits  $^{31.32}$ .

Both laboratory experiments (neutrino masses, proton decay, and so on) and astrophysical observations (more precise measurements of both the large and small scale structure of the 3 K background, further studies of the distribution of galaxies, and so on) should have a great impact on theories of galaxy formation in the next few years. During the workshop, it was clear that this was already beginning to occur. Silk discussed how the recently observed quadrupole anisotropy in the 3 K background tightly constrains both the adiabatic and isothermal pictures<sup>33</sup>, and Davis reported on the recent observation of large holes in space (devoid of galaxies)<sup>34</sup>. These holes are most easily explained in the adiabatic theory, while the observed quadrupole asymmetry favours the isothermal theory.

#### Exotic relics from the big bang

As the energies explored by manmade machines (<1,000 GeV) are quite modest in terms of the energy scales of GUTs ( $\geq 10^{14}$  GeV), the early Universe is the only laboratory where physics at such energies can be studied. However, because this accelerator has not been in operation for  $\sim 10,000$  Myr, to study the physics of such high energies the observationalist must often search for fossil evidence although energies of up to  $10^{19}$  GeV were probably realized in the early stages of the hot big bang. One example that received a lot of attention at Santa Barbara is the search for relic supermassive magnetic monopoles.

Polyakov and 't Hooft<sup>35,36</sup> have shown that whenever a simple group (most of the gauge groups proposed for grand unification are simple groups) spontaneously breaks down to a group which contains a U(1) factor [for example, to the low energy symmetry group, SU(3)×SU(2)×U(1)] magnetic monopoles with a mass of order 100 times the symmetry breaking scale exist. These monopoles are topological objects associated with 'kinks' in the Higgs field (the Higgs mechanism is used to break the symmetry spontaneously). It is difficult to see how less than ~1 monopole per horizon volume would be created, as causality precludes straightening out the Higgs field on larger scales. (The horizon volume is the largest-sized region that could have communicated by light signals since the 'bang'.) The usual symmetry-breaking scheme of GUTs results in a predicted relic

monopole abundance of about one per nucleon<sup>37</sup>. As these monopoles have a mass of  $\sim 10^{16}\,\mathrm{GeV}~(\sim 10^{-8}\,\mathrm{g})$ , it is not surprising that such a present abundance can be ruled out by using our rather meagre knowledge of the present mass density of the Universe ( $\rho \leq 4 \times 10^{-29}\,\mathrm{g}\,\mathrm{cm}^{-3}$ , which corresponds to  $\Omega \leq 2$ ). A monopole abundance of one per nucleon would correspond to  $\Omega \cong 10^{14}$ .

This discrepancy between prediction and observation has been widely discussed. Possible solutions discussed at the workshop included mechanisms that would confine monopoles and antimonopoles in much the same way that quarks are confined in nucleons. In such schemes the confined monopoles and antimonopoles can annihilate to an acceptable level. Another possible solution is to keep the symmetry from being restored at high temperatures, as the monopoles are produced when the symmetry breaks. Finally, the most popular solution seems to be a hypothesis in which the universe supercools thereby delaying the GUT phase transition (for example, the inflationary universe of Guth<sup>38</sup>). Delaying the phase transition reduces the number of monopoles produced, as the horizon volume grows with time and only about one relic monopole is produced per horizon volume. As no suppression mechanism yet proposed seems compelling, the number of monopoles present today, if any, is hard to determine. Clearly some observational information would be useful.

Past searches for magnetic monopoles have usually looked for the ionization caused by the passage of a monopole through matter. Because the monopoles of GUTs are expected to be heavy and slow, the past experimental limits do not apply. At Santa Barbara, Cline outlined his proposal to search for supermassive magnetic monopoles at a huge steel plant in Mt Iron, Minnesota. Cline believes that the relic monopoles that have bombarded the Earth since its formation became bound to ferromagnetic domains. Heating iron ore above the Curie point destroys the domains, thus releasing the monopoles. The perfect place to do the experiment is at the Mt Iron processing plant run by United States Steel, where there is a pipe which carries 18 million tons per year of iron-ore above the Curie point, releasing their pent-up monopoles to be detected as they fall in the Earth's gravitational field. Results of such searches will be awaited by both particle physicists and cosmologists. The discovery of a magnetic monopole would be direct evidence for grand unification and the spontaneous symmetry breaking of the GUT.

On a much smaller scale, but just as fundamental to both particle physics and cosmology, is the continued claim of the observation of fractional charge by a low temperature experimental group at Stanford headed by Fairbank<sup>39</sup>. The importance of this experiment to particle physics is obvious, but cosmologists also have a stake in the results. Just as the monopoles were the fossil remains of the early Universe at temperatures of 10<sup>15</sup> GeV, fractional charge may be the remnant of the period in the early Universe when hadrons were formed out of the quark gas.

The most obvious interpretation of the Fairbank experiment is the existence of free, fractionally-charged quarks. However, it is generally believed (but not rigorously proved) that in the SU(3) colour gauge theory of quarks and gluons, coloured objects (for example, quarks) must always be bound into colourless states which have integral charge. For this reason the Stanford experiment has worried particle physics theorists. One possibility is that SU(3) is also spontaneously broken, but at a very small energy scale, ~10-50 MeV (refs 40, 41). This allows for the existence of free coloured objects, like quarks. Other possibilities exist: the Stanford group could be observing leptons (which are not coloured) with non-integral charge, or perhaps they are seeing bound states of quarks and other integrally charged coloured objects which would result in fractionallycharged, colourless objects. Unfortunately, as particle physics does not supply a unique model to explain the existence of fractionally-charged states, cosmologists cannot give a unique answer for the expected abundance from production in the early Universe. Wagoner reported that the relic abundances predicted from various models range from uncomfortably large to undetectably small<sup>42</sup>.

#### Phase transitions

During the 30 or so orders of magnitude of thermal history of the Universe  $(T \sim 10^{32} \text{ K} \rightarrow 3 \text{ K})$  many phase transitions associated with spontaneous symmetry breaking (SSB) should have taken place<sup>43</sup>, including the SSB of the GUT, of SU(2)×U(1), and perhaps the SSB of other intermediate symmetry stages. During these phase transitions it is likely that interesting phenomena occurred: during the GUT transition baryosynthesis and monopole production, and in all the stages of SSB, the possible production of entropy, creation of structure (that is, primordial density fluctuations), and generation of a cosmological term.

The standard mechanism (or crutch) for SSB is the Higgs mechanism. The details of the SSB transition depend on the parameters of the Higgs sector of the theory. If the transition is first order, there may be a large entropy release, which if it occurs after baryosynthesis dilutes the baryon asymmetry. (The baryon asymmetry is more correctly quantified as the baryon-to-entropy ratio rather than the baryon-to-photon ratio; however, today the two are essentially equivalent as most of the entropy in the Universe is in the 3 K background.) In the simplest model of SSB (Coleman-Weinberg symmetry breaking) it has been shown that the large entropy release associated with the SSB of  $SU(2) \times U(1)(T \sim 300 \text{ GeV})$  would dilute the baryon asymmetry produced during baryosynthesis by an unacceptably large factor, that is, leading to  $B \le 10^{-11}$  today<sup>44-46</sup>.

More intriguing are the possible cosmological effects associated with the vacuum energies which result from SSB. The vacuum energy associated with the Higgs condensate corresponds to a uniform energy density which acts like a cosmological term (A), and which charges by  $0(T_c^4)$  during a phase transition which occurs at temperature  $T_c$ . We know that the cosmological term today is not significant,  $(\Lambda/8\pi G) \le 4 \times 10^{-29} \text{ g cm}^{-3} \cong 10^{-46} \text{ GeV}^4$ . It seems that unless one specifies an initial compensatory, 'true' cosmological term to cancel the 'induced' ones, the Universe would be left with an enormous cosmological constant due to SSB. Guth has discussed the so-called inflationary universe, in which the cosmological term temporarily dominates the dynamics of the Universe<sup>38,47</sup>. An initial compensatory cosmological constant,  $\Lambda_i/8\pi G \sim 0(T_c^4)$ , is initiated to cancel the induced cosmological term associated with the GUT phase transition  $(T_c \sim 10^{15} \text{ GeV})$ . If the phase transition proceeds slowly, then the cosmological term  $\Lambda_i$  will soon begin to dominate the dynamics and will do so until it is cancelled when the transition has been completed. During this period the Universe undergoes a de Sitter phase in which the expansion is exponential<sup>47</sup>. The de Sitter phase has several potentially beneficial effects. First, the horizon (distance over which light signals can propagate) is inflated to encompass most of the observed Universe today, whereas in the usual FRW model, the horizon at the GUT epoch contains only 1 baryon. This could explain why on the largest scales the Universe is so smooth, as a patch of the Universe which could have been smoothed by particle interactions before the de Sitter phase is inflated to an enormous size during this epoch. The inflated horizon might also allow for the production of large-scale (galactic size and larger) density fluctuations which are necessary for galaxy formation. As mentioned earlier, the large horizon might possibly provide a solution to the monopole problem. When the transition has been completed, there is a large entropy release (baryosynthesis proceeds after this). This could explain the Universe has expanded so long without recollapsing or becoming negative—curvature dominated (in units of the fundamental time unit,  $t_{\rm Planck} \sim 10^{-43} \, {\rm s}$ ,  $\tau_{\rm Universe} \sim 10^{60} \, {\rm t_{Planck}}$ ). The curvature term grows in importance with time; as it is not significant today, earlier it must have been far less significant than the energy density term. Why this is the case is a mystery (the so-called 'flatness problem'). However, the tremendous entropy release would provide a natural explanation because it

allows the energy density to increase relative to the curvature term by a very large factor. Guth's 'inflationary scenario' is not without its complications. First, there is the problem of gracefully returning to FRW behaviour; and second, it presupposes the reality of these enormous induced cosmological terms associated with SSB. The cosmological term today is essentially zero; perhaps we should adopt the point of view that it has always been zero (for reasons we do not understand).

#### Fundamental problems

The 6-month program and week-long intensive workshop at Santa Barbara were both evidence that cosmology and particle physics is a young field that has come of age. As we have tried to stress here, the symbiotic relationship which exists between these two fields is one in which information flows in both directions: cosmology can constrain particle physics theories and particle physics can have profound implications for cosmology. Cosmological constraints restrict the class of viable theories, rather than provide guidance or direction. However, there is the hope that particle physics theories, GUTs in particular, might help to resolve some of the fundamental problems of classical (that is, non-quantum) cosmology. To conclude, we shall try to assess the impact to date and the possible future impact of GUTs on what we consider to be the six fundamental puzzles of classical cosmology<sup>48</sup>.

The most significant contribution of grand unification is baryosynthesis. Although we are still a long way from being able to predict the precise magnitude, or even the sign of the baryon asymmetry with GUTs, for the first time a framework exists for understanding why there is a baryon asymmetry when the laws of physics at the microscopic level are so nearly symmetrical.

The introduction of GUTs to cosmology, while appearing to have resolved one puzzle, unfortunately seems to have created another: the monopole problem. All the straightforward GUT scenarios of the early Universe predict that relic, superheavy monopoles ( $M \ge 10^{16}$  GeV) should today provide enough mass density to close the Universe many times over ( $\Omega \ge 10^{14}$ ). Perhaps, the solution is already at hand; however, it is equally likely that the resolution of the problem will be more subtle, and may even come at a very fundamental level with other important implications. The detection of relic monopoles and the determination of their cosmic abundance would be of the greatest significance for both particle physics and cosmology.

Another fundamental problem is the 'flatness' problem<sup>49</sup> which can also be regarded as an 'age' problem. There is only one fundamental time scale:  $t_{\rm Planck} \sim 10^{-43}\,\rm s$ ; the age of the Universe is  $\sim 10^{60}\,t_{\rm Planck}$ . In newtonian language, the 'flatness' problem is: the initial (at  $t_{\rm Planck}$ ) kinetic and potential energies of the Universe must have been equal (and opposite) to an accuracy of some 50 or so decimal places, otherwise the Universe would have long since recollapsed or entered a coasting phase (dynamics dominated by negative curvature). Guth's 'inflationary universe' may answer this problem, but at present there are other serious problems inherent to the scenario.

Next, there is the homogeneity problem: on the largest scales (≥10 Mpc) the Universe is very smooth, while on smaller scales it is quite lumpy (stars, galaxies, clusters, and so on). In the standard FRW cosmology, the large-scale homogeneity cannot be explained by causal processes, because causally distinct regions are uniform to a high degree of accuracy. Regions of the sky separated by more than ~1° or so were causally disconnected when the microwave photons we detect today were emitted, and yet have temperatures which are equal to within -1 part in 103. Given the 'proper spectrum' of initial small inhomogeneities (which is also a matter of disagreement), the present structure can be understood as being a result of the growth of the small fluctuations due to gravitational instability. The origin of the initial inhomogeneities is still a mystery which is also exacerbated by the existence of particle horizons in FRW models. The creation of fluctuations at very early times (for example, during a phase transition) is severely limited by the size of the horizon, and for this reason the resulting spectrum of

perturbations is usually far too small to be of any importance. Again, Guth's scenario may help to resolve both parts of the homogeneity problem, as the horizon problem is effectively circumvented.

GUTs thus far have shed no light on why the present cosmological term is so small. As is well known, quantum field theories (for example, quantum electrodynamics) predict an infinite energy density associated with the vacuum, which results in an infinite cosmological constant. (The Casimir effect strikingly demonstrates the reality of the vacuum energy density.) With a spontaneously broken gauge theory the situation is even more perplexing as the vacuum energy density changes during each stage of SSB. Even if one chooses to ignore the infinite contribution to the cosmological term, one must face the induced cosmological constants associated with each stage of SSB. If an initial compensatory, 'true' cosmological term is introduced to cancel the induced ones, then to cancel the cosmological constant which results from the SSB of the GUT, the initial cosmological constant must be specified to an accuracy of >100 decimal places. It is the assumption of the reality of these induced cosmological terms associated with SSB that leads to Guth's 'inflationary universe'. It is perhaps equally reasonable to suppose that as the cosmological term is essentially zero today, it has always been zero (for an unknown reason). It seems clear that a satisfactory solution to the cosmological constant problem will involve connections between cosmology and quantum field theory at a very deep level.

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GUTs have also had very limited impact on the isotropy problem: why is the Universe isotropic to such a high degree of precision? The isotropy is evidenced by the 3 K background, galaxy counts, and the X-ray background.

The solution to some or even all of the five unresolved conundrums may well involve quantum gravity. As was discussed by Hartle, Hu and Parker at the Santa Barbara workshop, there are indications that particle creation due to quantum gravitational effects is highly efficient in damping initial anisotropy<sup>50</sup>, and that quantum gravitational effects may remove particle horizons<sup>51-54</sup> from cosmological models, perhaps leading to a solution to both parts of the horizon problem. However, given the magnitude and fundamental nature of the problem of formulating a quantum theory of gravity, it seems most prudent not to pin one's hopes on quantum gravity, but to pursue solutions that do not involve quantum gravity. It is not implausible that GUTs could provide solutions to most of these five puzzles.

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# ARTICLES

# No evidence of rings around Neptune

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Observations of two stellar occultations by Neptune were used to search for possible equatorial and polar rings. No ring occultation events were identified, and an upper limit of 0.07 can be placed on the optical depth of any equatorial rings greater than 5 km wide with radii greater than 31,400 km. Any ring system of Neptune must be much less extensive than the ring systems of Uranus and Saturn, but a jovian-type ring of low optical depth would have escaped detection by our search. THE unexpected discovery<sup>1</sup> of rings around Uranus in 1977 excited new interest in planetary rings and the extreme narrowness of its nine rings—some only a few kilometres wide in the radial direction—created a new puzzle for dynamicists<sup>2,3</sup>. The interest increased in 1979, when a ring system was found around Jupiter<sup>4</sup>. The three known ring systems appeared strikingly different: Jupiter's, of extremely low optical depth; Saturn's, broad and bright; and the uranian rings, dark and narrow. Voyager 1 established an important similarity between the saturnian and uranian rings, when its high resolution images revealed that a multitude of narrow saturnian rings constitute the several broad rings that we see from Earth<sup>5</sup>.

### Rings around Neptune?

Reports of visual detection of rings around Neptune, made shortly after the planet's discovery in 1846, have been either withdrawn or unconfirmed<sup>6</sup>. Furthermore, the question of whether Neptune has rings cannot be answered on theoretical grounds, as the origins of the three known ring systems have not been established and may all be different<sup>7-12</sup>. One might suspect that because three of four giant planets have rings, Neptune should also—assuming a ring system results directly or indirectly from the formation of a giant planet. On the other hand, the three planets with rings also have regular satellite systems, but Neptune's satellite system is peculiar<sup>13</sup>. It has only two confirmed satellites: Triton, a large satellite with an inclined retrograde orbit, and Nereid, a distant satellite with a highly eccentric orbit.

#### **Techniques**

The most sensitive techniques for detecting planetary rings have been Voyager imagery and stellar occultations<sup>14</sup>. Since Voyager 2 is not due to explore the Neptune system until 1989, we have selected from lists of possible occultations by the Neptune the most appropriate events for detecting a ring system. Using occultations to search for rings around Neptune is more difficult than using these to observe the uranian rings, because an equatorial ring system around Neptune would subtend an angle of only 2 arcs perpendicular to the motion of Neptune; the uranian rings subtend an angle of 8 arcs perpendicular to the motion of uranus. Furthermore, a complete search for rings around Neptune must include the possibility of rings in the non-equatorial, yet stable, orbits described by Dobrovolskis<sup>15</sup>. The only previously reported occultation observations are those of Nicholson and Jones 16, who obtained incomplete data for an occultation on 21 August 1980. They describe a possible ring event (at a projected equatorial radius of 1.5  $R_N$ ), but there have been no corroborating data.

The two occulations used in our search were (1) star no. 28 in the list of Mink et al.<sup>17</sup> which occurred on 10 May 1981, and (2) an uncatalogued star that we identified as an observable event only a few days before it occurred on 24 May 1981. As shown in Table 1, the first event was observed from various sites around the Pacific Ocean, while the second was observed only from Cerro Tololo. Observations were made at several wavelengths

Table 1 Ring search observations								
Date	Observatory	Telescope† aperture (m)	Filter*	Observers				
10 May 1981	Mauna Kea	3.0 2.2 0.6	$R$ $B, R_1$ $R_2$	Elhot, Mink Liller, Pingree Franz, French				
10 May 1981 10 May 1981	Siding Spring Mount Stromlo	1.0 1.9	_	Dunham, Zarro Jones, Nicholson				
24 May 1981	Cerro Tololo	0 <b>8</b> <b>4.</b> 0	B, R <sub>1</sub>	Baron, Smith Elias, Martin				

<sup>\*</sup>The filters had the following centre wavelengths and passbands (FWHM): B, 0.44 and 0.10  $\mu$ m,  $R_1$ , 0.78 and 0.18  $\mu$ m;  $R_2$ , 0.87 and 0.06  $\mu$ m; K, 2.2 and 0.4  $\mu$ m.

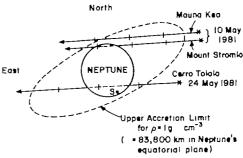


Fig. 1 Regions probed for rings around Neptune. The solid lines show the tracks of the occulted stars relative to Neptune as seen from Mauna Kea, Mount Stromlo and Cerro Tololo. The tracks begin at 11 00 UTC for the 10 May event and at 2:00 UTC for the 24 May event. The tic marks on each track occur at 30-min intervals. The dashed ellipse shows the upper accretion limit <sup>22</sup> in Neptune's equatorial plane. Inside this limit, the tidal force from Neptune exceeds the gravitational attraction for spherical particles of equal size. Here, such particles could not accrete into larger bodies, but would remain as rings (see note added in proof).

with equipment described elsewhere 18-20. In each case the observers recorded (on magnetic tape) the intensity of starlight as the star passed behind the region around Neptune that could accommodate rings.

#### Results

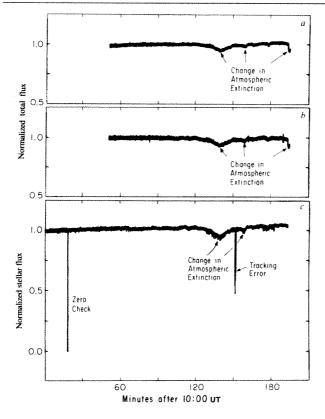
Figure 1 shows a plot of the tracks of the stars as they would appear in the sky, the dashed portions indicating where Neptune occulted the star. The duration of the Neptune occultations, along with the occultation radius of Neptune obtained from Taylor's circular solution<sup>21</sup>, were used to establish the positions of the tracks with respect to Neptune. Also shown is the upper accretion limit for material of  $\rho = 1~{\rm g~cm^{-3}}$  in the equatorial plane of Neptune. Within the upper accretion limit, the gravitational attraction of spherical bodies of equal size is less than the tidal force from Neptune<sup>22</sup>. Hence, spherical particles of equal size would remain as rings, and not condense into satellites, inside this limit. For particles of unequal size, the accretion limit would be closer to Neptune. The direction of Neptune's pole in Fig. 1 was assumed to coincide with the pole about which Triton's orbit precesses<sup>23,24</sup>. Our assumed direction could differ by a few degrees from the actual direction of Neptune's pole, which cannot be calculated because Triton's mass is uncertain. The sky-plane velocity of the star was 19 km s<sup>-1</sup> for the first event and 22 km s<sup>-1</sup> for the second.

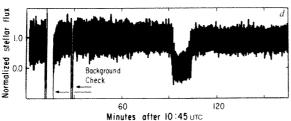
The two events combined cover the space for rings around Neptune. Fortunately for this search, Neptune did not occult the star as seen from Mauna Kea, so that this track covers the

Table 2 Regions probed for rings

UTC on 10 May 1981		ected equa istance (kr Siding Spring	UTC on 24 May 1981			
10.00	182,200	190,900	190,900	2:00	130,900	
10.30	138,300	146,000	146,000	2:30	77,300	
11.00	97,900	102,800	102,700	3:00	34,300	
11:30	67,900	65,100	64,700	3:30	52,800	
12:00	64,700	48,500	47,800	4:00	104,100	
12 30	91,300	70,500	69,700			
13 00	130,400	109,900	109,300			
13.30	173,800	154,100	153,400			

Projected equatorial distances for times other than those tabulated should be obtained from three-point interpolation of the tabulated values. Figure 1 can be used to determine when the star was behind the planet and we could not probe for rings; Fig. 2 shows the time intervals when data were being acquired. Minimum equatorial distances are. MKO, 61,700 km at 11:48 UTC; SSO, 48,400 km at 11:58 UTC; MSO, 47,600 km at 11:58 UTC, and CTIO, 31,400 km at 3:08 UTC.





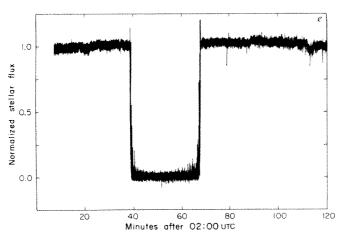


Fig. 2 Intensity versus time for: a-c, the 10 May event from Mauna Kea; d, the 10 May event from Mount Stromlo; e, the 24 May event from Cerro Torolo. All data have been averaged at 0.2s. Frames a, b show the combined light from Neptune and the star observed simultaneously at 0.44 µm and 0.78 µm with the 2.24-m telescope. Frame c shows the intensity at 2.2 µm observed with the IRTF. All notable dips in signal can be ascribed to causes other than ring occulations. In d the times of immersion and e immersion of the star behind Neptune were used to calculate the apparent position of the star relative to neptune. In e the large increases in signal at immersion and emersion by Neptune are the occultation spikes, caused by density variations in the atmosphere by Neptune. The dip in signal at 3:18:41 UTC is too narrow to have been caused by a ring occultation. The remaining conspicuous dips could not have been caused by continuous equatorial rings, or corresponding dips would have been observed during the 10 May occultation.

possibility of polar rings. For equatorial rings, Table 2 gives the projected distance from the centre of Neptune in its equatorial plane, as a function of time, for all four stations. The minimum distance probed was from Cerro Tololo, just as the star emerged from behind the planet (at a point corresponding to 31,400 km [1.3  $R_N$ ] in the equatorial plane).

The observed intensities versus time are shown in Fig. 2 for Mauna Kea, Mount Stromlo and Cerro Tololo. The averaging time was chosen to match the width of a diffraction-limited occultation profile, which would have been produced by a ring narrower than  $\sim 4.6$  km (refs 25, 26). Note that the star occulted on 10 May had a projected diameter at Neptune of  $\sim 10$  km (inferred from *JHK* photometry), which would broaden any ring occultation profiles to about twice their diffraction limit.

First we examine the data obtained with the 2.24-m telescope and IRTF (NASA's 3.0-m IR telescope facility), displayed in Fig. 2 a-c. As the spectra of Neptune and the star are not similar, the relative contributions of each are quite different at the three wavelengths observed. For the blue channel, the contribution of the star is <1% of the total signal; for the red channel, the star contributes 21% and for the 2.2-µm channel the stellar contribution is >99%. Hence, a partial occultation of the star by material near Neptune would not be detectable in the blue channel, and would have different fractional drops in the red and 2.2-µm channels. On the other hand, a change in atmospheric extinction (assuming it was not wavelength dependent) would cause approximately the same fractional drop in total flux for all three channels. A third possible cause of a drop in signal, a guiding or tracking error by one of the telescopes, would appear in the data from one telescope.

All observed drops in signal have explanations other than occultations by possible rings. These have been marked in Fig. 2. The small dip in the red channel near 11:20 UTC occurred during a focusing adjustment of the telescope. The remaining dips in the IRTF signal are <5% of the stellar intensity. Hence, we conclude that any rings of Neptune within the region probed from Mauna Kea, (>5 km wide in the sky plane plane projection) must have an optical depth <0.05.

The data from the 0.8-m telescope at Mount Stromlo, shown in Fig. 2d, contain the occultation that we used to calculate the path of the star with respect to Neptune. Although the data are noisier than those obtained from Mauna Kea, the star as seen from Mount Stromlo probed closer to Neptune, and these data would have revealed any rings similar to the  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\varepsilon$  rings of Uranus<sup>14</sup>. The 2.2-µm data from the 1.9-m telescope at Mount Stromlo (not displayed) have much better signal-to-noise ratios than those obtained with the 0.8-m telescope, and nine dips in signal occurred in these data that were candidates for ring occultations. However, all these events happened at times when corresponding events would have been visible from Mauna Kea as well, if they were caused by rings. The lack of corresponding events at Mauna Kea leads us to conclude that the events recorded with 1.9-m telescope were caused by thin clouds and/or poor seeing. If any had been due to a total occultation by a small satellite, a corresponding event would have been recorded with the 0.8-m telescope. No total occultations, except for Neptune, were recorded (see Fig. 2d).

The data from the occultation by Neptune on 24 May 1981 are shown in Fig. 2e. The increases in signal above full-scale at planetary immersion and emersion are the spikes caused by density variations in Neptune's atmosphere. As this event was observed with only one telescope, and at a single wavelength, we had more difficulty in establishing the cause of dips in signal. The most conspicuous dip occurred at 03:18:41 UTC, which corresponds to an equatorial radius of 1.55  $R_{\rm N}$ . Curiously, this is near the same equatorial radius of the possible ring occultation reported by Nicholson and Jones<sup>16</sup>. However, we believe both events had causes other than rings and their coincidence is fortuitous. At high resolution the CTIO event has a FWHM of only 2.7 km. This is about half the minimum width (4.6 km, due to diffraction<sup>25,26</sup>) that could have been produced by a ring or small body at the distance of Neptune. Hence we conclude that

this dip was not caused by a ring or small body near Neptune. The other three notable dips in signal that occur after 3:25 UTC were apparently not caused by continuous equatorial rings either, as this region was probed during the 10 May event, with negative results. In fact, the dip at 3:25 UTC occurred when the planet was near the edge of the photometer beam. Other than the dips just discussed and the occultation by Neptune, the signal never drops below 0.93 of its unocculted value. Hence, we place a limit of 0.07 on the optical depth of any equatorial rings of Neptune, with widths > 5 km and radii > 31,400 km.

Our upper limit is about four times lower than would have been necessary to detect the least opaque uranian ring (ring 6), but we could not have detected a jovian-type ring, of low optical depth. (The jovian ring has not yet been detected by radio or optical occultation<sup>27,28</sup>.) Hence, in terms of detectability by occultations, any system of rings around Neptune must be much less extensive than the uranian system.

In terms of the amount of material that could exist as neptunian rings, our limit on the optical depth allows a large range of masses. On one hand, we could have detected as little as 10<sup>12</sup> g of material concentrated into a narrow ring. However, the form of the material least amenable to detection by our observations would be a collection of small satellites. ~1 km in diameter. This diameter would be too small for an individual satellite to cause an observable occultation. If the mean optical depth of the collecton of satellites were < 0.07, we would not have detected the ensemble either. If the material were carbonaceous ( $\rho = 3 \text{ g cm}^{-3}$ ), the combined mass of these satellites could be as great as  $4 \times 10^{23} \text{ g}$ . This is comparable to recent upper limits on the mass of Saturn's rings<sup>29</sup> and would equal that of a single satellite, 600 km in diameter.

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#### Conclusions

It is puzzling to reconcile our failure to detect rings with the recent discovery (through occultation observations) of an apparently small, third satellite of Neptune30, with an orbital radius of  $3 R_N$ . As the odds against discovering a lone body in this manner would be so great, one might conclude that Neptune has several, if not many, small satellites. One small satellite orbits just inside, and another just outside, the F ring of Saturn; at least two exist near Jupiter's ring and several small satellites have been postulated to be intimately associated with the uranian rings.

Hence we must still entertain the possibility of a neptunian ring system that we were unable to detect. A further opportunity to search for rings with occultations, with better sensitivity, occurs<sup>17</sup> in 1983, and Voyager encounters Neptune in 1989. If these opportunities fail to reveal rings, then we need wait only ~ 108 yr until tidal interaction between Neptune and Triton will have caused Triton to move close enough to Neptune to break up into what should be a spectacular system of rings<sup>3</sup>

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Note added in proof: An error of  $2\frac{1}{3}$  was found by P.D.N. in Smoluchowski's expression for the upper accretion limit<sup>22</sup>. For  $\rho = 1 \text{ gm cm}^{-3}$ , the correct radius of the upper accretion limit in Fig. 1 is 66,500 km (not 83,800 km).

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# Global satellite measurements of water vapour, wind speed and wave height

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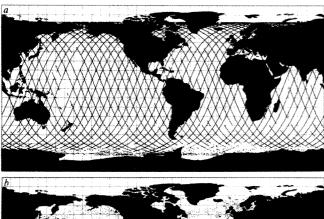
Data from the 100 days of Seasat observations in 1978 provided the first global maps of mean wind speed and wave height measured from satellites. They reveal previously unknown features in both fields and demonstrate the potential for satellite monitoring and forecasting of the worldwide sea state.

ON 28 June 1978 the United States launched Seasat, an experimental satellite to demonstrate the utility of microwave remote sensing of the ocean surface. On board were three active microwave radars (an altimeter, a scatterometer and a synthetic aperature radar) and two passive radiometers (a scanning multichannel microwave radiometer and a visible and IR radiometer). Because of their relative insensitivity to cloud cover comparedqith visual and IR sensors, microwave sensors have the advantage of all weather operations. A more detailed description of the Seasat mission is given in ref. 1. Although a short circuit in its electrical system ended the observations

prematurely on 10 October 1978, Seasat provided the first global pictures from space of wave height and wind speed. Seasat also measured atmospheric liquid water, water vapour, sea-surface txperature and sea-surface topography with better accuracy than from previous satellites.

#### Measurements

Results of global measurements of atmospheric water vapour by the Seasat Scanning Multichannel Microwave Radiometer (SMMR) and wave height and wind speed by the Seasat altimeter (ALT) are presented here. The primary objective of ALT



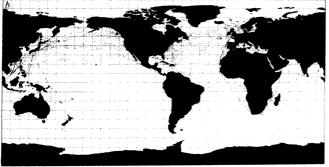


Fig. 1 Representative 3-day global data coverage by: a, the Seasat altimeter; and b, conventional ship weather reports (courtesy of D. McLain, National Marine Fisheries Service, Monterey, California). Both the examples shown are for 9-11 August 1978.

was to measure the height of the sea surface relative to a reference ellipsoid. However, the shape and power of the returned radar pulse are also used to infer wave height and wind speed at the sea surface. The 13.5-GHz Seasat altimeter is a successor of the GEOS 3 altimeter with a shorter pulse width (3.125 ns) and higher repetition rate (1,020 Hz) which lead to more precise measurements of sea surface height (better than 10 cm). The 1.59° half power beam width directed at the spacecraft nadir gives a cross track sea-surface footprint that increases from 2.4 km for 0 m wave height to 11.6 km for 20 m wave height. For the 1-s averages presented here, the along track sea-surface footprint ranges from 9.4 to 18.7 km. For typical wave heights of 3 m the ALT footprint is  $\sim 5 \times 12$  km.

ALT operated intermittently, producing data over a total of 78 days. Because Seasat circled the Earth about 14 times each day at an altitude of ~800 km and an inclination of 108°, successive equatorial crossings moved in a westward direction with a separation of ~2,500 km. In 3 days, the equatorial spacing of the ground tracks was about 900 km. The full Seasat mission resulted in global coverage with ~135 km equatorial spacing of ground tracks. Typical 3-day ALT data are shown in Fig. 1a. The gaps in the data are due either to ALT being temporarily turned off or to a flagged error condition (such as anomalously high wave height or wind speed estimates or a satellite tilt angle beyond 0.5°). For comparison, global data from conventional ship weather reports are shown in Fig. 1b for the same 3-day period: ALT provides more than an order of magnitude increase in the quantity of data (131,700 compared with 7,600 observations). Even more importantly, the spatial coverage by ALT is more nearly uniform over the globe than the ship reports which tend to be concentrated along standard shipping routes in the Northern Hemisphere.

A detailed description of how the wave height can be extracted from the ALT radar return pulse is given in refs 2, 3. The waves stretch the leading edge of the returned radar pulse because of early returns from wave crests and later returns from wave troughs. Thus, the slope of the leading edge of the returned radar pulse is inversely related to the wave height. The shape of the returned pulse has been empirically related to the significant wave height (SWH) defined to be four times the r.m.s. of the crest-to-trough wave height, roughly equivalent to the average

height of the one-third largest waves present in the ALT footprint. Computations of wave heights were carried out on board the satellite providing real-time wave measurements. Two simple bias corrections were later applied on the ground: a pre-launch calibration bias removal and a bias correction due to satellite tilt angle (flagged if  $>0.5^{\circ}$ ). A scatter plot comparing 51 buoy measurements with the ground-processed ALT estimates of SWH is shown in Fig. 2a (see ref. 3). The mean and r.m.s. differences (ALT minus buoy) over this sample data set are small (28 and 38 cm respectively). Apparently, the on-board processor estimates of SWH > 2 m may be biased  $\sim$ 50 cm high but the present sample size is too small to confirm this.

The altimeter antenna measures specular return from the nadir sea surface which is primarily determined by that part of the ocean wave spectrum with wavelengths longer than the -2-cm wavelength of the incident radiation. As the wind speed increases, the sea-surface roughness increases and a greater fraction of the incident radiation is scattered away from the satellite. Thus the power of the returned radar pulse is inversely related to the wind speed. A more detailed description of the relationship between radar backscatter and the wind speed is given in ref. 4. A scatter plot comparing 87 buoy measurements with ALT estimates of wind speed is shown in Fig. 2b (see ref. 3). A direct comparison of Seasat and GEOS 3 altimeter measurements during the overlap of the two satellite missions indicated that the Seasat backscatter values were biased 1.6 dB higher than GEOS 3 (see ref. 3). The present wind estimates were generated from the Seasat ALT backscatter values by first removing this 1.6-dB bias and then using a wind speed algorithm previously derived for the GEOS 3 altimeter (see ref. 3). The mean and r.m.s. differences (ALT minus buoy) from this limited sample size are 0.25 m s<sup>-1</sup> and 1.58 m s<sup>-1</sup> respectively. The lack of data means that the reliability of the relationship at wind speeds in excess of 10 m s<sup>-1</sup> is questionable. However, experience with GEOS 3 indicates that an altimeter can provide reliable wind speed estimates to at least 20 m s<sup>-1</sup>. Investigations are underway to determine the accuracy of the Seasat ALT wind speed estimates over a broader range of conditions and from a larger data base.

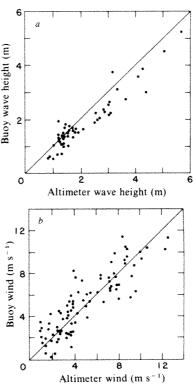


Fig. 2 a, Comparison of significant wave height estimated by the Seasat altimeter against 51 buoy measurements. b, Comparison of wind speed estimated by the Seasat altimeter against 87 buoy measurements. Data from ref. 3.

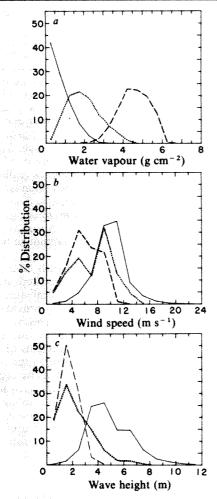


Fig. 3 Distributions of a, water vapour; b, wind speed; and c, wave height estimates from Seasat for the 5° lat. bands centred at 52.5° N (dotted curves), 2.5° N (dashed curves) and 52.5° S (solid curves). All observations between 7 July 1978 and 10 October 1978 are included.

An additional measurement required in any precision altimetric mission is the integrated atmospheric water vapour between the sea surface and the satellite. Water vapour estimates are necessary to make pathlength and attenuation corrections to the ALT radar pulse. On Seasat, integrated water vapour was estimated using the near nadir cell of the SMMR. The presence of water vapour between the satellite and the sea surface increases the atmospheric index of refraction which increases the effective radio frequency path length by 10-50 cm. The altimeter pathlength correction was estimated directly by a linear function of the SMMR brightness temperatures at 18, 21 and 37 GHz (both horizontal and vertical polarization). These pathlength correction estimates were then converted to total integrated atmospheric water vapour based on comparison with direct water vapour measurements from radiosondes. A detailed discussion of this procedure is given in ref. 5.

#### Cover picture

The 3.5-month Seasat climatological averages of integrated atmospheric water vapour, wind speed and wave height are shown on the cover. More than 3.5 million observations have been averaged globally into 2.5° square areas. The typical number of observations per 2.5° square is around 500 (higher at high latitudes where the satellite ground tracks converge). During the last month of the mission, Seasat was held in a locked orbit with 900-km equatorial spacing of ground tracks over an ~3-day repetition period. This resulted in an uneven sampling distribution over the 2.5° global grid in the 3.5-month averages presented here; 2.5° squares along the locked orbit ground track typically contained nearly twice as many sample data points over

the 3.5-month period as the 2.5° squares between locked orbit ground tracks. (The regions between locked orbit ground tracks were not sampled at all from 9 September to 10 October.) Consequently, to obtain useful pictures of water vapour, wind speed and wave height, the data had to be smoothed zonally with a 7.5° running mean filter (no smoothing meridionally), so the images on the cover effectively represent 3.5-month climatological averages over 2.5° lat. by 7.5° long. areas.

The average water vapour is shown in the upper panel. The highest values are in the tropics (especially the Asian monsoon region and the intertropical convergence zone in the eastern tropical Pacific) and the lowest are at high latitudes. The effects of cool dry air blowing equatorwards over the eastern ocean basins and turning westwards as the trade winds are also evident as well as the region of high water vapour in the southern intertropical convergence zone at about lat. 10° S in the western tropical Pacific. The anomalous observations close to Antarctica are believed to be due to the presence of ice producing increased microwave brightness temperatures.

The average wind speed measured by ALT is shown in the middle panel. Several classical features of the wind field are clear: the north-east and south-east trade winds separated by the doldrums (region of light winds) in both the Atlantic and Pacific Oceans; the horse latitudes (another region of light winds) separating the trade winds from the stormy westerlies in both hemispheres; the south-east trade winds in the south equatorial Indian Ocean and the high wind speeds from the summer monsoon in the northwestern Indian Ocean. The highest wind speeds are in the Southern Hemisphere winter storm region between 50°S and Antarctica (especially southwest of Australia). The narrow region of high wind speeds in the eastern tropical Pacific at about lat. 2° N between long. 90° W and 140° W is a feature previously not seen in ship wind reports which are very sparse in this region. Note also the jet of high wind speed off the coast of California (see ref. 6).

The lower panel shows the average significant wave height during the Seasat mission. This is the first accurate picture of global wave conditions and, we believe, the first reliable measurement by any method of ocean waves for winter season in the Southern Hemisphere. It reveals some interesting features. The average wave height is small in the summer Northern Hemisphere (generally 2-3 m) and the largest waves are located in the winter Southern Ocean where the wind speeds are highest (especially south-west of Australia where mean wave height is >5.5 m). The smallest waves are found in the western Atlantic and Pacific Oceans (average height <1.5 m) in regions of light winds. We expect that further analysis of the ALT wave height data will ultimately lead to a better understanding of the process of wind wave generation.

#### Discussion

The fact that the 2.1-m contour between 50° and 55° N in the North Pacific does not extend all the way to the coast can probably be explained by the coarse 2.5° lat. by 2.5° long, resolution of the data presented here. Examination of finer-resolution data in this region reveals that the apparent decrease in wave height in the 2.5° averages close to the coast is due to the presence of a distinct wave shadow behind Queen Charlotte Island (the largest of the many islands off the west coast of Canada). In the finer-resolution data, the 2.1-m waves extend all the way to the western shores of these islands but wave heights are <1 m behind the islands.

Note that ice in the ALT footprint causes very erratic behaviour in the shape and power of the returned radar pulse which typically results in rapid changes from very high to very low wave height estimates by ALT. This anomalous behaviour is flagged on the ALT geophysical data records gferated fromhhe sensor data by the Jet Propulsion Laboratory, thus providing a potential method for mapping ice coverage from altimeter measurements. Anomalously high wave height estimates have been excluded from our data so that wave height estimates over ice are small. Thus the ice-covered area is represented in the

wave height map on the cover by the light-coloured region close to Antarctica.

While climatological averages emphasize geographical differences within each field, they give no indication of the range of conditions likely to be encountered in a particular region at different times. The rather striking zonal banding of each of the fields suggests that latitudinal distributions can provide some initial insight into water vapour, wind speed and wave height variability during the Seasat mission. The probability distributions of each of the three fields for the three 5° lat. bands centred at 52.5° N, 2.5° N and 52.5° S are shown in Fig. 3.

The features evident in the water vapour distributions in Fig. 3a are not surprising. They clearly show the higher integrated water vapour levels found in the tropics. Only 2% of the observations in the equatorial band were <3 g cm $^{-2}$  while 96%of the observations in the high southern latitude band were <2 g cm<sup>-2</sup>. This reflects the lower water vapour saturation level of the cold Southern Hemisphere winter air. The cool high northern latitude band shows intermediate water vapour values with 75% of the observations between 1 and 3 g cm<sup>-2</sup>. A more detailed study of the water vapour estimates might be useful in climatological studies of the global time variability of the latent heat of vapourization transferred from the ocean to the atmosphere.

The wind speed distributions in Fig. 3b show some unexpected features. As the high southern latitude band is in the winter storm region, the higher wind speeds in this band are not surprising. Large variations might also be expected due to the passage of individual storms. However, the winds in this band seem to be relatively steady: 68% of the observations were between 8 and 12 m s<sup>-1</sup>. In comparison, the trade winds of the equatorial band, which are noted for their steadiness, show a peak at much lower wind speeds but a broader distribution: 76% of the observations were between 4 and  $10\,\mathrm{m\,s^{-1}}$ . Another curious feature is the probability distribution for the high northern latitude band: the range of wind speeds is broader than either of the other two bands but there are two pronounced peaks, a primary peak at 9 m s<sup>-1</sup> and a secondary peak at 5 m s<sup>-1</sup>. Present studies are aimed at determining whether the double peaks arise from zonal averaging of an inhomogeneous

field or whether they reflect a seasonal change in the wind speed distribution during the 3.5-month period.

The wave height distributions are shown in Fig. 3c. The equatorial band has the narrowest range of wave conditions: 70% of the waves were <2 m and virtually none were >4 m. In comparison, only 50% of the waves in the high northern latitude band were <2 m and 5% were >5 m. In contrast to both the equatorial and high northern latitude bands, the peak in the wave height distribution in the stormy high southern latitude band is at a much higher value (3.5 m as opposed to 1.5 m) and the range of wave conditions is much wider. Only 2% of the waves observed were <2 m and over 40% were >5 m. The secondary peak at 6.5 m is rather puzzling; it is difficult to explain in terms of wind forcing as there is no similar doublepeaked structure in the wind speed distribution in this latitudinal band. The double peaks probably result from zonal averaging of the large wave heights south and west of Australia with the smaller wave heights elsewhere in this 5° latitudinal band (see cover).

There is no way of knowing at present whether the 3.5-month average wind speed and wave heights presented here are typical for this time of year. However, these geophysical maps demonstrate the unique ability of satellites to provide global measurements of wind and wave conditions. This will be especially useful in data-sparse regions such as the Southern Hemisphere (see Fig. 1b) where present forecasts of weather and sea states are extremely unreliable. Even in the Northern Hemisphere, forecasting skill is limited in many regions by sparse data coverage over the oceans. An operational altimetric mission could improve worldwide sea state forecasting.

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# Dipoles of the $\alpha$ -helix and $\beta$ -sheet: their role in protein folding

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As a result of the regular arrangement of peptide dipoles in secondary structure segments and the low effective dielectric constant in hydrophobic cores, the electrostatic energy of a protein is very sensitive to the relative orientation of the segments. We provide here evidence that the alignment of secondary structure dipoles is significant in determining the threedimensional structure of globular proteins.

IT has long been known that in the  $\alpha$ -helix the alignment of the peptide dipoles parallel to the helix axis gives rise to a macrodipole of considerable strength (for review see ref. 1). With a dipole moment for each peptide unit of ~3.5 D, a helix of, for example, 10 residues has a dipole moment of 34 D, as 97% of the peptide dipole points in the direction of the helix axis. It has been shown<sup>2</sup> for points near the helix termini that the effect of the helix dipole is equivalent to the effect of half a positive unit charge at the N-terminus of the helix and half a negative charge at the C-terminus. The same authors provided evidence that the considerable electric field due to the helix dipole moment is used by proteins in: (1) binding negativelycharged groups, such as phosphate groups; (2) rendering protein side chains located near the N-terminus more nucleophilic; and, (3) stabilizing charged transition states or intermediates along the catalytic pathway. So far ~20 helices have been observed which bind negatively-charged groups near their N-termini. Another 10 proteins have their active site close to the Nterminus of a helix (W.G.J.H., unpublished results). For papain, an extensive quantum mechanical calculation3 showed that the field generated by the helix dipole is a major factor in the stabilization of the sulphhydryl-imidazole ion pair essential in the catalytic mechanism of this enzyme<sup>4</sup>. Secondary structure formation also seems to be influenced by side chain-main chain electrostatic interactions<sup>5,6</sup>.

Here we extend the role of the helix dipole to the area of protein folding. In this we are motivated by the idea that the directionally sensitive and relatively long-range dipole-dipole interaction is an important factor in ordering the secondary structure in the hydrophobic interior of folded globular proteins.

#### Model calculations of helix-helix interaction

Figure 1 shows, for a simple case, the result of model calculations of the electrostatic energy between the backbone dipoles of two helices as a function of their distance and relative orientation. The strongest variation in energy occurs when one helix axis is turned by  $180^{\circ}$  relative to the other (Fig. 1b). The energy difference between a parallel helix pair and an antiparallel pair is considerable (Fig. 1b,c). These calculations

also show that the interaction between two helix dipoles reaches an upper limit at a helix length of  $\sim 35$  residues (Fig. 1d). We consider first the extent to which proteins containing helices and no  $\beta$ -sheets reflect these simple electrostatic model considerations. Proteins containing  $\beta$ -structures will be discussed later.

#### All-helical proteins

In an elegant analysis of protein structures, Levitt and Chotia<sup>7</sup> showed schematically three major types of all-helical structures. By assigning 'plus' signs to the N-termini and 'minus' signs to the C-termini (indicating the effective charge) in their two-dimensional drawings, the alternating pattern of plus and minus signs tentatively confirms the trend of our model calculations.

Estimates of the actual three-dimensional interaction energies between the helices in the all-helical and predominantly helical proteins tobacco mosaic virus (TMV)<sup>8,9</sup>, myoglobin<sup>10</sup> and parvalbumin<sup>11</sup> are given in Table 1. These calculations show

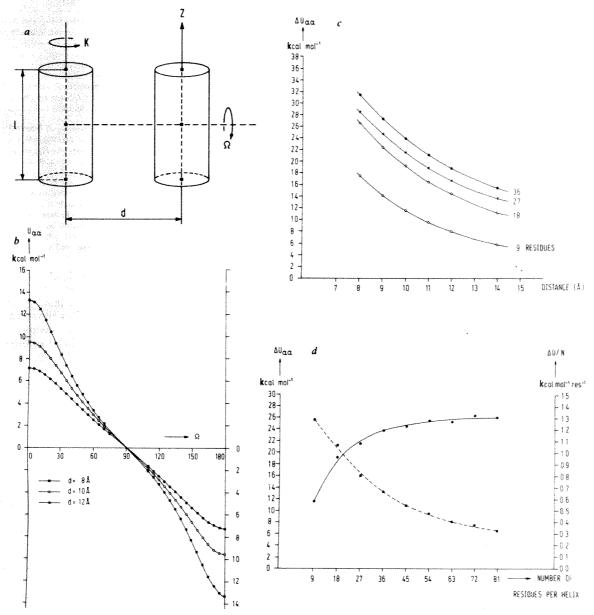


Fig. 1 a, The relative position and orientation of two helices in the simple case where the helix axes are perpendicular to the line connecting the centres. A helix can be rotated about its own axis by an angle K (with negligible variation in electrostatic energy) and about the inter-helical axis by an angle  $\Omega$  (with considerable variation in electrostatic energy) between two model  $\alpha$ -helices as a function of twist angle  $\Omega$ . The length of both helices is five turns, that is, 18 residues or 27 Å. c, The difference in electrostatic energy between a pair of parallel ( $\Omega = 0$ ) and a pair of antiparallel ( $\Omega = 180$ ) helices falls off gradually with increasing distance, d. d, The difference in electrostatic energy between a pair of parallel ( $\Omega = 0$ ) and a pair of antiparallel ( $\Omega = 180$ ) helices at a mutual distance of 10 Å as a function of helix (solid line). The figure illustrates that the difference becomes less important on a per residue basis for longer helices (broken line). For the model calculations, we used polyalanine in a helical geometry, generated by the program of Hermans et al. The only partial charges are those of the peptide group<sup>2</sup>: C, 0.42; O, -0.42; N, -0.20 and H, 0.20 unit charge. No cutoff radius was used and dielectric screening was neglected.

**Table 1** Calculated electrostatic interaction energies between the backbones of  $\alpha$ -helices and  $\beta$ -strands in various proteins

Protein	Type of protein	No. of $\alpha$ -helices	No. of β-strands	$U_{\alpha\alpha}$ (kcal mol <sup>-1</sup> )	$U_{lphaeta}$ (kcal mol $^{-1}$ )	Ref.
Met-myoglobin	$\alpha_{\mathbf{a}}$	9	***	-22.7	_	10
TMV	$\alpha_{\mathbf{a}}$	4		-14.3	-	8, 9
Parvalbumin	$\alpha_{\rm a}$	6	-	-11.8	. <del>.</del>	11
Cytochrome b <sub>5</sub>	$\alpha_*$	6	<b>-</b> ,	-4.7	****	22
Phospholipase A2	$\alpha_{\mathbf{a}} \beta_{\mathbf{a}}$	4	2	-8.3	-0.8	41
Cytochrome c	$\alpha_{\mathbf{a}}\boldsymbol{\beta}_{\mathbf{a}}$	4	3	-3.4	-1.4	42
Hen egg-white lysozyme	$\alpha_{\mathbf{a}}\boldsymbol{\beta}_{\mathbf{a}}$	6	5	-12.8	-1.6	19
Phage T4 lysozyme	$\alpha_{\mathbf{a}} \boldsymbol{\beta}_{\mathbf{a}}$	10	3	-23.5	-0.7	20
Thermolysin	$\alpha_{a}\beta$	7	14	-3.6	-5.4	21
Lactate dehydrogenase	$\alpha_{p}\beta_{p}$	9	15	+14.0	-14.0	43
Alcohol dehydrogenase	$\alpha_p \beta_p$	10	20	+14.2	-15.3	44
Glyceraldehyde phosphate dehydrogenase	$\alpha_{\mathcal{B}}\beta_{\mathfrak{p}}$	7	18	+5.8	-5.7	45
Adenylate kinase	$\alpha_{\beta}\beta_{p}$	10	5	• +4.9	-10.5	46
Rhodanese	$\alpha_{\mathfrak{p}} \beta_{\mathfrak{p}}$	10	10	+13.5	-10.3	32
Subtilisin	$\alpha_{p}^{p}\beta_{p}$	8	9	+5.2	-15.0	47
Dihydrofolate reductase	$\alpha_{p}\beta_{p}$	4	8	+3.2	-3.8	48
Flavodoxin	$\alpha_{p}\beta_{p}$	5	7	+13.3	-4.9	36
Triose phosphate isomerase	$\alpha_p \beta_p$	8	8	+6.6	-7.0	49
Carboxypeptidase	$\alpha_{p}\beta_{p}$	9	12	+11.4	-11.0	50
Papain/actinidin	$\alpha\beta + \beta_a$	6	8	+13.3	-6.6	51, 52

The table demonstrates the favourable alignment of  $\alpha$ -helices in all- $\alpha$  proteins and the compensation for unfavourable helix interactions by favourable helix-strand interactions in other proteins.  $U_{\alpha\alpha}$  is the sum of all main-chain dipole helix-helix interactions,  $U_{\alpha\beta}$  the sum of all main-chain dipole helix-strand interactions.  $U=\Sigma$  ( $qq_0/r_0$ ), where  $r_{ij}$  is the distance between two charges  $q_i$  and  $q_i$ , with no cut-off in r and with the partial charges? C=0.04.2, C=0.04.2, N=-0.20, C=0.04.2, N=-0.20, corresponding to a peptide dipole moment of 3.5 D in the direction of the N-H and O-C bonds. Indices i and j label the residues in the segments; i and j are not in the same segment. A dielectric constant was not used because of the breakdown on the atomic scale of continuum theories on which the concept of dielectric constant is based. This breakdown is illustrated quantitatively by molecular dynamics simulations<sup>53</sup>. The dielectric response, however, can be estimated and is physically due to the electronic polarizability of the protein atoms and the orientational polarizability of solvent water. The effects of the former can be separated into two contributions which occur in opposite directions. (1) Recent theoretical estimates of an effective dielectric constant for the non-polar atoms in the protein interior are  $\epsilon = 2$  (for example, derived from atomic polarizabilities<sup>54</sup>). This decreases U by a factor of  $\sim 2$ . (2) Cooperatively aligned backbone dipoles in both  $\alpha$ -helix and  $\beta$ -sheet mutually polarize each other, increasing the O+C and N+H dipoles by a factor of  $\sim 2$ . (2) Cooperatively aligned backbone dipoles in both  $\alpha$ -helix and  $\beta$ -sheet mutually polarize each other, increasing the other actions in the interior of a sphere of low dielectric surrounded by water: the screening is only effective near the surface and dies off quickly in the interior. As helices are usually nearer the protein surface than parallel  $\beta$ -sheets, this means that our  $U_{\alpha\alpha}$  values are an o

very favourable overall electrostatic interactions between the helices, in complete qualitative agreement with our model calculations. Although these energies are difficult to calculate precisely (see Table 1 legend), we do not expect any change in the qualitative conclusions when more accurate calculations are available.

The all-helical proteins myohaemerythrin<sup>12</sup>, ferritin13 cytochrome  $b_{562}$  (ref. 14) and cytochrome c' (ref. 15) strongly resemble the optimally antiparallel TMV structure16. The allhelical protein uteroglobin contains only antiparallel helices<sup>17</sup> The larger protein cytochrome c peroxidase 18 contains eight helices of which five form an antiparallel arrangement and none are aligned in parallel. Our model is also supported by the structure of 6-phosphogluconate dehydrogenase (M. Adams and S. White, in preparation): four pairs of antiparallel helices are wrapped perpendicularly around two long antiparallel helices which form the core of this protein. In addition, helices in the helical domains of hen egg-white lysozyme19, phage T4 lysozyme<sup>20</sup>, thermolysin<sup>21</sup> and cytochrome  $b_5$  (ref. 22) have a definite tendency to run antiparallel. This is reflected in the negative interaction energies  $U_{\alpha\alpha}$  calculated for these proteins (see Table 1).

#### Proteins with alternating $\alpha$ and $\beta$ structures

From an electrostatic viewpoint, it is surprising that in proteins with alternating  $\alpha$ -helices and  $\beta$ -strands, the helices are roughly parallel, that is, with seemingly unfavourable helix dipole alignment. In studying this phenomenon electrostatically, we examined the arrangement of peptide units in parallel  $\beta$ -strands. From schematic drawings of hydrogen bonding patterns in parallel  $\beta$ -sheets (Fig. 2), it is obvious that the N $\rightarrow$  H as well as the C $\leftarrow$  O dipoles point backwards with respect to the N- to C-terminal direction of the strands. This suggests that the

parallel  $\beta$ -sheet has a significant overall dipole moment, with the N-terminal end of the strands corresponding to the positive end of the dipole. Quantitatively, about one-third of the peptide dipole is parallel to the strand direction. This allows an approximate description of a parallel  $\beta$ -strand as having 1/15 of a positive charge near the C-terminus (Fig. 2a). Electrostatically, then, the  $\alpha$ - $\beta$  dipolar interaction is favourable when helices and strands are antiparallel.

A second aspect of the electrostatic interaction between helices and sheets concerns the twist of the sheet<sup>23</sup>. A consequence of this twist is that the 'parallel' helices in the  $\alpha/\beta$  proteins make considerable angles (typically  $-40^\circ$ ; ref. 24) with each other, thus decreasing appreciably the unfavourable interaction between their dipoles (Fig. 1b).

We have calculated the actual  $\alpha-\alpha$  and  $\alpha-\beta$  dipole interactions in various  $\alpha/\beta$  proteins and the simple considerations above seem to be valid generally (Table 1). The negative values for  $U_{\alpha\beta}$  qualitatively confirm our hypothesis of a favourable strand-helix interaction. This interaction compensates fully or partly for the unfavourable helix-helix interaction (Table 1). In our energy estimates, we disregarded the difference in electrostatic screening near the surface and in the interior. As the parallel  $\beta$ -sheets are sandwiched by the helices, we have overestimated  $U_{\alpha\alpha}$  relative to  $U_{\alpha\beta}$  and so the actual total energies are probably more favourable. Thus, domain structures with parallel central  $\beta$ -sheets antiparallel to surrounding helices may be significantly stabilized by electrostatic interactions.

## All- $\beta$ proteins

In globular proteins containing only  $\beta$ -sheets, there is an overwhelming tendency for the strands to align in an antiparallel manner (ref. 7 and Table 2). The same seems to be true for fibrous  $\beta$ -sheet proteins<sup>25,26</sup>. Electrostatically, an energy

difference between the two kinds of pleated sheet may be related to the fact that in the antiparallel sheet all peptide dipole moments seem to cancel each other out: there is no residual moment in either direction (Fig. 2b). In the parallel arrangement the components of the dipole moment parallel to the strand direction interact unfavourably with each other. A simple model calculation, assuming only a dipole moment in the parallel strand and no dipole moment in the antiparallel strand, shows that this effect may be  $\sim 0.4$  kcal mol<sup>-1</sup> for a pair of short  $\beta$ -strands containing four residues. This gives a difference in electrostatic energy of  $\sim 0.8$  kcal mol<sup>-1</sup> between a parallel and antiparallel arrangement of three strands, which is much smaller than the energy difference for  $\alpha$ -helices, but it may nevertheless be significant.

#### The $\alpha_a \beta_a$ and larger proteins

A small number of proteins do not fall into the all- $\alpha$ ,  $\alpha/\beta$  or all- $\beta$  categories, for example the C-terminal fragment of the ribosomal protein L7/L12 (ref. 27), in which the three helices and three  $\beta$ -strands are optimally antiparallel to each other. The L7/L12 protein is very stable<sup>27</sup>—probably due not only to the close packing of nonpolar residues in its unusually hydrophobic core, but also to the optimally antiparallel alignment of secondary structure backbones embedded within that core. Proteins like L7/L12 can be described as  $\alpha_a\beta_a$ , whereas the 'classical'  $\alpha/\beta$  proteins may be coined  $\alpha_p\beta_p$  (a, antiparallel; p parallel). The all-helical proteins may then be described as  $\alpha_a$ , and the all- $\beta$  proteins as  $\beta_a$ .

In larger proteins a mixture of folding patterns occurs, for example, in p-hydroxybenzoate hydroxylase<sup>28</sup>. This protein consists of three domains which can be described as  $\alpha_p \beta_p$ ,  $\beta_a$  and  $\alpha_a$ .

In non-compact interfaces between subunits in oligomeric proteins, dielectric screening by the solvent will be much greater than in hydrophobic cores. Hence, preferred orientations for  $\alpha$ -and  $\beta$ -dipoles from different subunits are expected to be less pronounced.

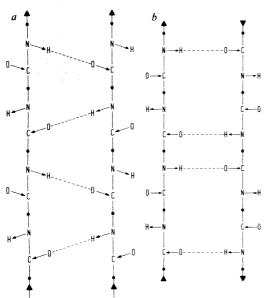


Fig. 2 Schematic representation of the arrangement of the  $N \rightarrow H$  and  $C \leftarrow O$  dipoles in  $\beta$ -sheets. a, Pair of parallel  $\beta$ -strands; b, pair of antiparallel  $\beta$ -strands. In parallel and antiparallel strands the  $N \rightarrow H$  and  $O \rightarrow C$  bonds have different directions relative to the strand direction. In the antiparallel case the bonds are fairly perpendicular to the strand direction, with little or no resultant dipole moment. In the parallel case, these bonds make angles of  $\sim 20^\circ$  with the N to C strand direction, thus about one-third of the peptide dipole moment runs parallel to the strand. Hence the component along the strand direction is  $\mu = 1/3 \, \mu$  (peptide) =  $1/3 \times 3.5 \, D = 1.15 \, D = 0.23 \, e Å$ . As the repeat distance along a  $\beta$ -strand is  $\sim 3.5 \, Å$  [refs 25, 76], a parallel  $\beta$ -strand can be considered as a dipole with a charge of +1/15 at its N-terminus and -1/15 at its C-terminus. Similar reasoning shows that the dipole of the  $\alpha$ -helix can be approximated by a charge couple of +1/2 and -1/2, respectively, at the N and C ends<sup>2</sup>. Thus on a per length basis, the  $\beta$ -parallel strand dipole is about a factor of five weaker than the helix dipole.

**Table 2** The predominance of antiparallel over parallel strand pairs in all- $\beta$  proteins and all- $\beta$  domains

Protein	Protein Strand pairs		Ref.
	$\beta_a$	$\beta_{ m p}$	
Elastase	11	0	59
Papain (domain II)	6	0	51
Ribonuclease S	3	0	60
Concanavalin A	12	0	61, 62
Rubredoxin	2	0	63
High-potential iron protein	2	0	64
IgG (Fab')	14	1	65
Prealbumin	5	1	66
Neurotoxin	4	0	67, 68
Superoxide dismutase	6	0	69
Bacteriochlorophyll protein	16	1	70
Subtilisin inhibitor	4	0	71
Soybean trypsin inhibitor	3	0	72
Tomato bushy stunt virus	14	0	73
Acid proteases	7	2	74
Total	109	5	

All- $\beta$  proteins here includes those with a negligible number of helices.

#### Charged side chains

In addition to the interactions between secondary structure dipoles in the protein interior, electrostatic interactions of charged side chains with each other and with the  $\alpha$ -helix dipoles are another component of the total electrostatic energy of a protein molecule. This component will, in most cases, be less important than the interactions between the  $\alpha$  and  $\beta$  dipoles. Experimental evidence for this is provided by chemical modification studies in which the charge of exposed residues is removed or even reversed while the catalytic activity of the proteins is little affected<sup>29-31</sup>. In addition, comparison of different amino acid sequences which form strikingly similar structures shows little similarity in the position of residues of similar charge (for example, rhodanese<sup>32</sup> and the serine proteases<sup>33</sup>). A likely explanation for this is effective electrostatic screening of charged residues due to counterions and solvent dipoles<sup>34,35</sup>.

Flavodoxin probably has a significant electrostatic interaction between charged residues and  $\alpha$ -helix dipoles<sup>36</sup>. In this protein, the charged residues are distributed non-uniformly with respect to the helices:  $\sim 10$  negative charges occur near the N-termini of the helices without any oppositely charged side-chain partner nearby. The helices in this  $\alpha/\beta$  protein interact quite unfavourably (Table 1), and in this case the large number of favourable charge-helix dipole interactions compensates, together with the favourable  $\alpha-\beta$  dipole interactions, for the unfavourable helix dipole interactions.

The tropomyosin dimer, which is not a globular protein but forms a coil of two very long parallel helices of 284 residues<sup>37</sup>, shows the predominance of a large number of salt bridges over the interaction between the helix dipoles. As McLachlan and Stewart observed<sup>37</sup>, the charged residues in this protein are situated in such a way that like charges clash when forming antiparallel helices, but as many as 30 salt bridges can be formed in the parallel arrangement. As the helix dipole interaction does not increase after ~30 residues (Fig. 1d), the charge-charge interaction is now the major factor in determining the direction of the helices with respect to each other. However,  $\alpha$ -helical homopolypeptides, which lack such charge pairs (for example, polyalanine) actually seem to prefer an antiparallel arrangement, both in fibres and in lamellar single crystals 26,38, which is in complete agreement with the results of our model calculations (Fig. 1).

#### Conclusion

If the values of the electrostatic energies given in Table 1 are compared with the experimentally observed values of  $12\pm5$  kcal mol<sup>-1</sup> for the free energy difference between folded and

unfolded protein conformations<sup>39</sup>, it seems that the interaction between the main chain dipole moments of secondary structure elements has a significant role in determining the conformation of a protein molecule.

Our electrostatic considerations are also in accord with the frequent occurrence of  $\alpha\alpha$ ,  $\beta\beta$  and  $\beta\alpha\beta$  units in proteins<sup>7</sup>. The picture which emerges is that the interior of globular proteins is a medium with low dielectric screening in which the interactions between secondary structure dipoles lead to preferred folding patterns. Kauzman<sup>40</sup> suggested that hydrogen bonds between peptide links and hydrophobic interactions are the most important factors in determining the overall configuration of

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globular protein molecules. It seems reasonable to suggest that relatively long-range electrostatic interactions in the hydrophobic protein interior should be added to this list.

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# Clustered arrangement of immunoglobulin $\lambda$ constant region genes in man

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The immunoglobulin  $\lambda$  light chain locus of man contains six  $\lambda$ -like genes arranged tandemly on a 50-kilobase segment of chromosomal DNA. The sequences of three of these genes correspond to three known non-allelic  $\lambda$  chain isotypes: Mcg, Ke<sup>-</sup>Oz<sup>-</sup> and Ke<sup>-</sup>Oz<sup>+</sup>. They surround a highly polymorphic and evidently unstable region that is repeatedly deleted when cloned in Escherichia coli. Three additional, but as yet unlinked, \( \lambda - like sequences have also been cloned, suggesting \) that the  $\lambda$  genes form an unexpectedly large family within the human genome.

THE formation of an active immunoglobulin light chain gene (k or  $\lambda$ ) involves the covalent joining of two distantly encoded segments of germ-line DNA<sup>1-5</sup>. In the case of the  $\kappa$  variable region of the mouse, V region diversity is accounted for largely by the availability of several hundred germ-line variable genes that can be joined to one of four active J region segments 8,9. The

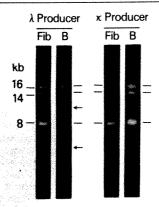


Fig. 1 Analysis of  $C_{\lambda}$  gene-bearing EcoRI fragments in human B-lymphocyte DNA. Light chain-expressing B cells (B) examined included monoclonal B lymphocytes, expressing  $\lambda$  or  $\kappa$  chain (as indicated) on their cell surface, taken from patients with high-count chronic lymphocytic leukaemia. Fibroblast (Fib) cell cultures served as germ-line controls for each individual. Genomic DNA was extracted from cells<sup>25</sup>, digested with EcoRI restriction endonuclease, size fractionated by agarose gel electrophoresis, transferred to nitrocellulose<sup>29</sup>, hybridized with  $^{32}$ P-labelled  $^{30}$  2.5-kb EcoRI-HindIII fragment obtained from the Hu $_{\lambda}$ 5 library clone (see Fig. 2 legend), and autoradiograms developed. DNA fragments identified in fibroblast DNA are indicated by thin lines; rearranged fragments in B-cell DNA by arrows. Identical results were obtained using as probe the subcloned 3.4-kb EcoRI-HindIII fragment obtained from the Hu $_{\lambda}$ 5 library clone (see Fig. 2).

precise cross-over point of this V-J joining can itself vary so as to create additional diversity around the site of recombination<sup>8-10</sup>. The arrangement of the human  $\kappa$  region gene is similar, but includes an additional active J region segment<sup>11</sup>.

The arrangement of mouse  $\lambda$  genes differs from that of  $\kappa$  genes in that the mouse  $V_{\lambda}$  repertoire is very small and only a single J region segment is likely to be encoded adjacent to each of the four known  $\lambda$  constant region genes 12.13. The human  $\lambda$  gene locus probably differs from that of the mouse as human antibody molecules are associated with a diverse population of  $\lambda$  chains, whereas mouse antibodies are not. Such a difference may reflect a much larger human  $V_{\lambda}$  gene repertoire. In addition, we have observed that there is a specific order of light chain gene recombination in which  $\kappa$  precedes  $\lambda$  14.15. The mechanism of this ordered rearrangement is unknown.

The human locus seems to be particularly favourable for examining the molecular basis for  $\lambda$  gene behaviour. The human  $\lambda$  constant region consists of at least four non-allelic forms that

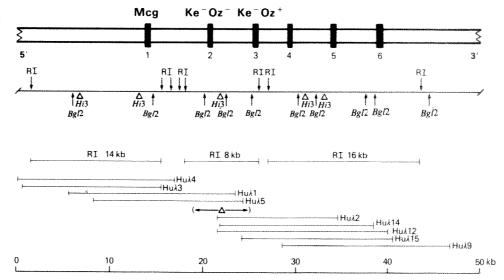
differ by limited amino acid substitutions to produce the serological markers Kern, Oz and Mcg (refs 16–19). Several additional  $\lambda$  variants have been recognized, but it is unknown whether these represent allelic variants or distinct isotypes<sup>20–22</sup>. Here we report the cloning and initial characterization of the germ-line  $\lambda$  constant region genes of man. These genes constitute an unexpectedly complex locus of at least six non-allelic genes that are arranged tandemly on a single chromosome. In addition, we have identified a polymorphic region within the locus that suggests a segment of particular genetic instability.

# Molecular cloning and identification of the human $\lambda$ constant region genes

To identify the human  $\lambda$  constant region genes, we used a mouse-human cross-hybridization strategy similar to that described for the identification of the human  $\kappa$  genes<sup>10</sup>. Our probe was a 0.24-kilobase (kb) MboII DNA restriction fragment derived from a mouse  $\lambda$ 1 cDNA clone (p- $\lambda$ -1; provided by A. Bothwell). This fragment corresponds to the gene segment that encodes amino acids 123 to 203 of the  $\lambda$ 1 constant region. The mouse  $\lambda$  constant region probe was used in conditions of low stringency to screen a human fetal liver library consisting of randomly cleaved DNA fragments cloned in bacteriophage  $\lambda$  CH4A (provided by T. Maniatis)<sup>23</sup>. In this way, seven distinct clones were obtained.

If the cloned fragments actually contained  $\lambda$  constant regions, we would expect them to hybridize to rearranged fragments of DNA in λ-producing B cells. We therefore examined DNA derived from  $\lambda$  light chain-bearing B lymphocytes obtained from a patient with high-count chronic lymphocytic leukaemia. (The leukaemic expansion of a lymphocyte is effectively monoclonal.) Subcloned fragments derived from one of the library clones were used as probes (Fig. 1). To control for possible polymorphic differences in the arrangement of hybridizing DNA fragments<sup>24</sup>, DNA extracted from fibroblast cultures established from the same individual served as a germ-line-like control. As shown in Fig. 1, the cloned probe detected two rearranged fragments in the DNA from the  $\lambda$ -producing B cells, whereas the DNA extracted from control  $\kappa$ -producing B cells demonstrated no such rearrangement. Note that the monoclonal λ-producing cells show rearrangement of two DNA fragments and that the 8-kb germ-line fragment has been lost in these cells. The most likely interpretation of this result is that rearrangement has occurred on both homologous chromosomes and that these rearrangements have occurred within or 3' to the 8-kb EcoRI fragment (see map, Fig. 2).

Fig. 2 Physical map of the human  $\lambda$  light chain constant region genes. arrangement of a cluster of six  $\lambda$  constant genes (filled boxes) along chromosomal DNA is shown at the top. The orientation is 5' to 3', left to right. A detailed map for the restriction enzymes EcoRI, HindIII and BglII is shown. The map was deduced by overlapping a series of library clones isolated from a human fetal liver DNA 3 and comparing cloned, genomic EcoRI fragments from that locus. Single and double restriction enzyme digestion patterns for EcoRI, HindIII, Bg/II and CfoI were compared and analysed for the presence of  $\lambda$  gene sequences by Southern Nine representative library clones (out of a total of 13) are shown. The library clones fell into two represented by clones 4, 3, 1, 5 and clones 2, 14, 12, 15, 9. Library clones that spanned the region indicated by  $\Delta$  invariably deleted extensive regions during propagation in the bacterial host. The unambiguous linkage between the two



groups of library clones was obtained by demonstrating that the restriction fragments generated from the cloned genomic 8-kb EcoRI fragment were identical to the sum of restriction fragments generated from an EcoRI-HindIII subclone of  $Hu\lambda 5$  plus a HindIII-EcoRI subclone of  $Hu\lambda 2$ . The library clones are represented by thin lines and designated as  $Hu\lambda$ . The genomic EcoRI fragments (RI) are represented by thin lines and their fragment lengths given. The three most 5' genes correspond to the non-allelic forms Mcg, Kern $^-Oz^-$  and Kern $^-Oz^+$ , as determined by nucleotide sequence analysis (see Fig. 4).

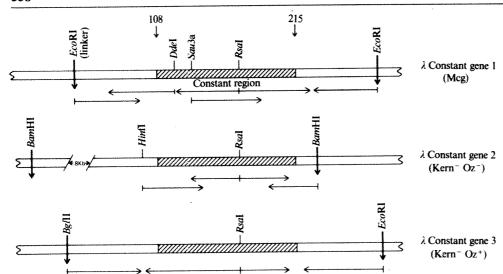


Fig. 3 Strategy for sequencing three human  $\lambda$  constant region genes. The coding regions of the three most 5' human  $\lambda$  constant region genes (genes 1-3, Fig. 2) were sequenced by the technique of Maxam and Gilbert<sup>31</sup> using the using the restriction sites indicated. The DNA restriction fragments used for (indicated bold bv sequencing arrows) were isolated preparatively on polyacrylamide gels as described previously 10. 32P ends are represented by short vertical lines and the extent and direction of sequencing are indicated by arrows. Hatched areas indicate coding regions (amino acids 108-215 of the  $\lambda$  polypeptide).

# Six λ-like constant region genes are arranged along a 50-kb segment of chromosomal DNA

Using the same human DNA fragments as probes, we rescreened the human clone library and obtained 19 distinct library clones (7 of which were identical to those obtained with the mouse probe, described above). In addition, the 8-, 14- and 16-kb genomic EcoRI fragments identified in Southern blots of genomic DNA (Fig. 1) were cloned directly using a fragment purification approach described previously<sup>25</sup>. By ordering the overlapping clones from restriction endonuclease site mapping data and Southern blot analysis (see Fig. 1 legend), we constructed the linkage map of the  $\lambda$  constant genes shown in Fig. 2. The map extends over 50,000 base pairs (bp) and contains at least six  $\lambda$  gene-like sequences tandemly arranged along the chromosome. The restriction enzymes EcoRI, HindIII, BglII and CfoI were used to establish the map. Thirteen of nineteen library clones overlap this locus; the remaining six clones are as yet uncharacterized. Six regions within this locus demonstrate strong homology by hybridization to the mouse  $\lambda$  constant region probe (data not shown). Note that these regions are regularly spaced along the chromosome ~5,000 bp apart.

# Identification of three additional $\lambda$ -like sequences

The order of genes (Fig. 2) allows us to predict that a V-J recombination event involving either gene 2 or 3 (on the 8-kb EcoRI fragment) should result in the deletion of DNA 5' to this fragment, in this case the 14-kb EcoRI fragment encoding gene 1. That this did not seem to occur in the  $\lambda$ -producing cell line used here (see Fig. 1) provided a clue that  $\lambda$ -like genes were encoded on two different 14-kb EcoRI fragments, one that fits into this map, and a second that does not. The non-allelic nature of the second fragment was confirmed by cloning it from two individuals and then showing that these separately cloned fragments, although identical to one another, were different from the originally cloned 14-kb fragment at several restriction sites (HindIII, BglII and CfoI, data not shown). We have not yet established the linkage relationship of the second fragment to the six clustered genes (P.A.H. and G.F.H., unpublished data). Indeed, careful examination of the  $\lambda$ -producing B cell in Fig. 1 shows a reduction of ~50% in intensity of the 14-kb fragment. This is consistent with the idea that both allelic copies of the 14-kb fragment encoding gene 1 have been deleted while both allelic copies of the second 14-kb fragment have been retained. In addition, we have cloned 16-kb and 5-kb Eco RI fragments of germ-line DNA that exhibit weak hybridization to the human and mouse  $\lambda$  constant region probes. Neither of these has been linked to the major six-gene cluster (G.F.H., P.A.H. and P.L.,

unpublished results). Whether these represent pseudogenes or functional coding segments is unknown.

# Sequenced genes correspond to specific human isotypes

To identify the  $\lambda$ -like regions (indicated in Fig. 2) positively as human  $\lambda$  constant genes, the nucleotide sequences of the three most 5' genes were determined using the sequencing strategy

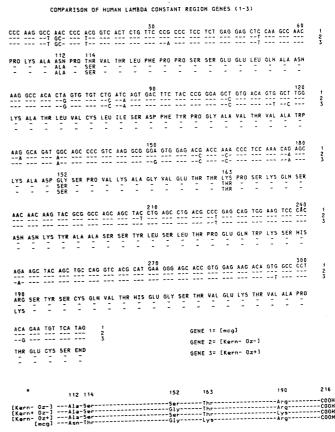
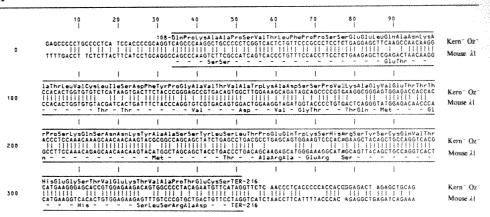


Fig. 4 Nucleotide sequences of three human  $\lambda$  constant region genes. The nucleotide sequences of the coding regions of the three most 5' genes (genes 1-3; see Fig. 2) are aligned. A dash in the nucleotide sequences of genes 2 and 3 indicates that the nucleotide in that position is identical to that in gene 1. The amino acid sequences deduced are shown below the nucleotide sequences. A dash in genes 2 and 3 indicates identity in that amino acid position to the gene 1 sequence. The amino acid sequence differences (positions 112, 114, 152, 163 and 190) between the four non-allelic forms of the human  $\lambda$  constant region are indicated at the bottom  $^{32}$ .

Fig. 5 Direct comparison of the nucleotide sequences of the human Kern  $Oz^-\lambda$  constant gene sequence and the mouse  $\lambda 1$  constant region sequences. The amino acid sequences deduced from the gene sequences are also shown. A dash in the mouse sequence indicates that the amino acid position is identical to that found in the human sequence. The frameshift mutation discussed in the text is at positions 253-270.



shown in Fig. 3. The nucleotide sequences corresponding to the amino acid coding regions are compared in Fig. 4 with their respective amino acid sequences.

At the protein level, at least four non-allelic forms of the human λ constant region have been identified <sup>16-19</sup>. The amino acid sequence differences between these are shown at the bottom of Fig. 4. By comparing these sequences with the amino acid translation of the three genes sequenced, we identify them as Mcg, Kern Oz and Kern Oz (Fig. 4). Presumably, one of the other genes—4, 5 or 6—corresponds to Kern Oz. The remaining two genes may represent additional non-allelic forms, may be separately encoded genes that are identical in their amino acid sequences to one of the four known non-allelic forms, or they may be pseudogenes. Direct nucleotide sequence analysis will distinguish between these possibilities.

#### **Evolutionary considerations**

The extremely close homology shown by the three sequenced genes suggests that they are the result of recent gene duplications. Single nucleotide differences are scattered throughout their coding regions, the fewest being in the carboxy terminal-coding portions. For example, genes 1 and 2 are identical in the 90 3'-terminal nucleotides of their coding sequences. There are four amino acid substitutions between genes 1 and 2 and 11 third-base silent mutations. Genes 2 and 3 differ by eight silent bases but only by a single amino acid substitution.

At the amino acid level, the human Kern Oz constant region is 72% homologous to the mouse  $\lambda$ 1 constant region, but only 61% homologous to the mouse λ 2 constant region. Therefore, it is reasonable to assume that the human genes have a more recent common ancestral antecedent with the mouse  $\lambda 1$  gene. To establish the nucleotide sequence relationships between the human and mouse  $\lambda$  constant genes, we have compared the Kern  $Oz^-$  nucleotide sequence with the  $\lambda 1$  constant region sequence of the mouse (Fig. 5)<sup>2,26</sup>. Strong homology extends throughout the coding region with single base differences being rather uniformly scattered. In the 315 nucleotides that comprise the coding block, 80 base substitutions have occurred. Thus the overall homology within the coding block at the nucleotide sequence level is 75%, which is greater than the homology observed between mouse and human k constant sequences . Base substitutions have occurred 19 times in the first position, 17 times in the second position and 44 times in the third position of amino acid codons, suggesting that selection occurs primarily on the basis of amino acid sequence. Curiously, one of these alterations has involved a compensating pair of single nucleotide insertion-deletion differences (positions 253 and 270; Fig. 5). A sequence characteristic of an RNA splice acceptor, PyPyXPyAg<sup>27</sup>, has been conserved in each sequence immediately 5' of the coding block.

# Restriction fragment length polymorphisms in the human $\lambda$ gene locus

During the isolation of cloned DNA sequences that spanned the region centred about the second and third genes (Ke^Oz^ and Ke^Oz^) we observed that these clones invariably deleted

extensive segments of DNA during propagation in the bacterial host. For this reason, our library clones fell into two groups that were difficult to link (represented by clones 4, 3, 1, 5 and 2, 14, 15, 9; Fig. 2). In fact, the definite linkage of these two groups had to be established by linkage of each to the cloned 8-kb EcoRI fragment (Fig. 2). Thus, there seemed to be an unstable sequence associated with this region at least as recognized by the bacterial host (designated by  $\Delta$  in the central 8-kb EcoRI fragment; see Fig. 2).

It was of interest to know whether this segment might also be unstable in man. Therefore, we prepared DNA from 15 individuals and used in situ hybridization to determine the arrangement of this fragment in this human population (Fig. 6). Even in such a small sample, we identified at least two, and possibly a third, arrangement of the  $\lambda$  constant genes within this fragment.

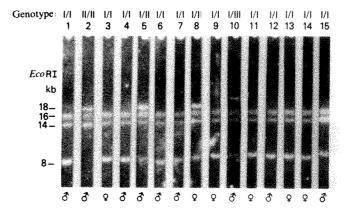


Fig. 6 Restriction fragment polymorphisms in the human C<sub>k</sub> locus. DNA was prepared from white cells isolated from 20 ml of blood taken from 15 individuals as follows: the cells were washed twice in ice-cold phosphatebuffered saline (PBS) and pelleted at 1,000 r.p.m. for 10 min. Red cells were lysed by adding 40 ml AKC lysing buffer (0.155 M NH<sub>4</sub>Cl, 0.01 M KCl) and left on ice for 10 min. White cells were pelleted at 1,500 r.p.m. for 10 min. DNA was extracted by resuspending the cells in 4 ml of 5% citric acid and breaking them with 10 strokes in a Dounce homogenizer (B pestle). Nuclei were pelleted by centrifugation at 2,000 r.p.m. for 5 min, then resuspended in 4 ml of 5% citric acid, layered over a 2-ml cushion of 0.88 M sucrose in 5% citric acid, and pelleted at 2,500 r.p.m. for 8 min. Nuclei were resuspended in 2 ml RSB (10 mM NaCl, 10 mM Tris pH 7.4, 25 mM EDTA) and diluted to 15 ml in RSB. Nuclei were pelleted and resuspended in 3 ml RSB. Proteinase K was added to a final concentration of  $10~\mu g~ml^{-1},~SDS$  to a concentration of 1%, and the mixture incubated at 37  $^{\circ}C$  for 2.5 h. The mixture was diluted to 5 ml in 0.5 M NaCl, extracted with phenol/chloroform/isoamyl alcohol (100:100:1) and centrifuged at 5,000 r.p.m. for 5 min. The aqueous phase was then extracted with ether, and precipitated by adding 2.5 vol ethanol. DNA was resuspended in 5 ml of 0.1 × SSC, digested with RNase (50 µg ml<sup>-1</sup>; 37 °C for 30 min), adjusted to 0.5 M NaCl, phenol and ether extracted as before, and ethanol-precipitated. The DNA was resuspended in 10 mM Tris pH 7.8, 5 mM NaCl, 0.5 mM EDTA and stored at 4 °C. Twenty ml of blood yielded ~400 µg of DNA. DNA (10 µg) was digested with EcoRI restriction endonuclease, size fractionated by agarose gel electrophoresis, transferred to nitrocellulese, hybridized to a human  $\lambda$  constant region probe (combined 1.2-kb BamHI-EcoRI fragment containing the Kern Oz C, gene and 0.8-kb BamHI-HindIII fragment containing the  $Mcg C_{\lambda}$  gene (see map, Fig. 2)), and autoradiographed. The lengths of the DNA fragments are indicated alongside. Three gene configurations were noted (types I, II and III) and are described in the text.

These polymorphisms represent differences in the lengths of the restriction fragments on which the λ constant genes Ke<sup>-</sup>Oz<sup>-</sup>(2) and Ke Oz+(3) reside. Case 1 is the most common genotype (type I), characterized by 8-, 14- and 16-kb EcoRI restriction fragments. Case 2 has a different genotype-in which an 18-kb fragment replaces the 8-kb fragment. Indeed, case 2 is homozygous for the 18-kb fragment as no 8-kb fragment is present in this individual. Case 5 represents an individual who is heterozygous for these alleles (type I/II). The allelism of these fragments has been further established by familial studies (P.A.H., G.F.H. and P.L., unpublished results). There is a third polymorphism (case 10, type III) associated with a 21-kb EcoRI fragment. As we have only one example of this type and because it retains at least one 8-kb fragment, we cannot be certain that the 21- and 8-kb fragments are allelic. The band intensity (Fig. 6) suggests, however, that this is the case.

Obviously these polymorphisms could arise either by point mutations or by rearrangements of large fragments of genomic DNA. The former mechanism seems less likely because it would require the alteration of several EcoRI sites to create an 18-kb (or larger) EcoRI fragment from the 8-kb fragment seen in type I (see map, Fig. 2). Rather, the labile behaviour of this fragment in E. coli suggests that it contains a 'hot spot' that readily deletes a segment of DNA. This, together with the frequent evolutionary rearrangement of DNA in higher organisms24,28, makes the rearrangement model a more attractive one.

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#### Multiple $J_{\lambda}$ - $C_{\lambda}$ regions and $J_{\lambda}$ repertoire

We have already noted that there are several important differences in the germ-line arrangements of  $\kappa$  and  $\lambda$  genes, a main one (noted in the mouse) being that while there are at least four active J regions available for V-J recombination for the single  $\kappa$  region gene<sup>4,5,8,9</sup>, there is only one J for each of the mouse  $\lambda$  genes so far characterized 12,13. A priori, this would limit the diversity that could be generated through V-J recombination in the  $\lambda$  system if there was only one  $\lambda$  constant region. The complexity of the human and mouse  $C_{\lambda}$  loci suggests a mechanism that may compensate for this deficiency. Instead of creating a strip of J regions in front of a single constant region (as seen in the  $\kappa$  locus), the entire  $\lambda$  region in the mouse has been amplified to create four J regions, each associated with its own C region. The net effect is to make four J regions available in both the  $\kappa$  and  $\lambda$  systems. In the human  $\lambda$  gene system, the options for using each constant region may similarly depend on V-J recombination, but the complex organization of the locus allows us to consider subsequent recombination steps (akin to heavy chain switching) or even alternative modes of RNA processing as mechanisms for activating other  $J_{\lambda}-C_{\lambda}$  segments. The complete characterization of this locus and precise identification of its J regions will allow us to distinguish among these possibilities.

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# Radio jet of 3C273

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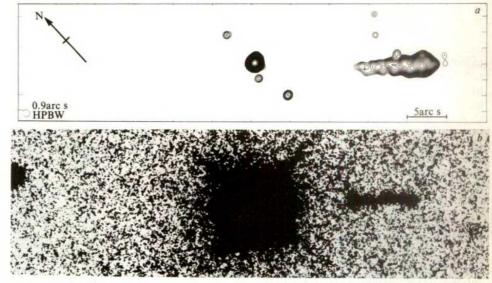
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Most radio sources are two-sided, but a minority appear onesided, 3C273 being the first-known and brightest example. There is no agreement<sup>1-4</sup> on whether such sources are intrinsically one-sided, or are normal double sources, one half of which is hidden by Doppler effects. We report here new radio observations at 408 MHz of 3C273 which show that the brightness of the postulated counter-jet is <1/100 of the brightness of the visible jet. If this ratio is due to Doppler beaming, the source must be seen almost end-on, and the whole jet must be moving at a quasi-relativistic speed (>0.7c) into an ambient medium with number density <0.6 m<sup>-3</sup>. Because several arguments suggest that such a density is implausibly low, the jet of 3C273 cannot be identified with the radio lobe of a normal double source. If the emitting regions are moving slowly the ejection from the nucleus is certainly to one side only. An alternative possibility is that the jet is moving relativistically and behaves as one of the fast beams in the beam-model of Blandford and Rees5.

New radio observations of 3C273 at 408 MHz (λ0.735 m) made with the MTRLI<sup>6,7</sup> at Jodrell Bank are shown in Fig. 1a, along with a reproduction to the same scale (Fig. 1b) of an optical photograph<sup>8</sup>, taken in the blue-green. (The optical QSO is 13th magnitude, and has a redshift of 0.158.) In neither picture is there any evidence of a counter-jet, and from the radio map a limit of <1:100 may be put on the brightness ratio of the two sides. The lack of significant emission either at radio or optical wavelengths in the region out to 12 arcs. from the quasar is suggestive of an invisible beam5 of material with ordered relativistic motion or of photons carrying energy to the outer component. However, we cannot rule out an alternative explanation of the 'gap', namely that the output power of the quasar has decreased significantly during the past  $\sim 10^5$  yr.

The radio emission in Fig. 1a shows 'wiggles' or oscillations from side to side of the central ridge-line, a phenomenon first suggested by Conway and Stannard9, the general features of whose map we confirm. The wiggles are not accompanied by the oscillations in brightness which would be expected if they were due to precession of the source axis. We suggest that they are more probably hydrodynamic instabilities 10 of the jet as it traverses the intergalactic medium.

Table 1 lists some observed parameters of the radio emission, which yield physical quantities that can be compared with predictions from different source models, in particular the model with a relativistically moving counterjet, which is hidden by Doppler beaming. We have calculated the magnetic field and Fig. 1 a, The MTRLI map of 3C273 at 408 MHz (A0.735 m) tilted so that position angle 223° is horizontal. The contour levels are logarithmic, with three contours to a factor 2 in brightness, the lowest contour being 0.12 Jy per beam. The HPBW of the beam is shown as a circle 0.9 arc s in diameter in the bottom left corner. The 3% sidelobes north and south of the central quasar are artefacts due to calibration uncertainties, and may be ignored. b, Photograph by Arp (with the same scale as a). Seeing is about 1 arcs. Neither a nor b show any evidence for a counter-jet, and display a gap in emission out to 12 arc s. from the central nucleus. The optical jet appears to have an approximately constant brightness along its length with a possible knot of emission nearest the quasar,



whereas the radio brightness increases outwards by at least 100:1. The radio emission also displays 'wiggles' along the length of the tail. If  $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ , 1 arc s equals 1.85 kpc.

energy density in the head from the spectral index and brightness using standard synchrotron theory11. If there is equipartition of energy between the magnetic field and relativistic particles (the latter term divided equally between ions and electrons) and if the bulk motion is zero, the field B is 29 nT and the energy density  $u_{\rm obs}$  is  $680\,{\rm pJ\,m^{-3}}$ . Multifrequency measurements of the radio polarization of the jet (R.J.D., unpublished data) show that the emission depolarizes at  $\lambda \approx$ 0.73 m. If this depolarization is due to internal Faraday rotation by thermal matter<sup>20</sup>, then the electron density  $n_e$  within the jet is 11 m<sup>-3</sup>. (This value is unusually low. If the depolarization is external,  $n_e$  must be lower still.) The sound speed, equal to  $(\frac{1}{3}$  energy density/matter density)<sup>1/2</sup>, is calculated to be 0.29c. Such a value means that the jet must be confined sideways by some means, because if it were in free expansion, the opening angle of the jet would be much larger than is observed. This conclusion is not altered if the motion of the jet is relativistic.

VLBI measurements<sup>12</sup> have shown the presence of 'superluminal' proper motions in the nucleus of the quasar. According to the ballistic model (see ref. 2) the most probable value of  $\theta$ , the angle to the line of sight, is related to the true velocity  $\beta_n c$  by:

$$\beta_n = \cos \theta$$
 (1)

This condition also results in the maximum enhancement of proper motion<sup>2</sup>. If equation (1) is satisfied, and if Hubble's constant  $H_0$  is  $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$  (a value indicated by recent work<sup>13,14</sup>), then  $\theta \approx 11^{\circ}$ ,  $\beta_n = 0.983$  and the Lorentz factor  $\gamma_n = 5.4$ . We assume that the jet also lies at  $\theta = 11^{\circ}$ , in which case the length D from the nucleus to the outer head is 200 kpc. (If the 'light-echo' model is adopted<sup>15</sup>, then  $\theta = 22^{\circ}$  and D = 100 kpc.)

We assume that the forward speed of the jet is  $\beta c \leq \beta_n c$ , with Lorentz factor  $\gamma \leq \gamma_n$ . The blueshift factor is

$$\eta = \frac{\nu_{\text{observed}}}{\nu_{\text{emitted}}} = \frac{1}{\gamma (1 - \beta \cos \theta)}$$
 (2)

It can be shown<sup>2</sup> that the energy density in a moving jet needed to produce the observed brightness is

$$u_{\text{rest}} = u_{\text{obs}} \eta^{-(2+\alpha)4/7}$$
 (3)

The forward motion of the jet will be opposed by a ram-pressure force proportional to the number density  $n_{\rm m}$  of the medium in front of the head. For relativistic speeds the ram-pressure, referred to the rest frame of the head, is

$$P_{\rm ram} = n_{\rm m} \mu (\gamma \beta c)^2 \tag{4}$$

where  $\mu$  is the average mass per particle in the ambient medium. The motion will be decelerated unless  $P_{\text{ram}} \simeq \frac{1}{3} u_{\text{rest}}$ . From these equations,  $n_{\text{m}}$  may be found from the measured brightness as a function of the forward speed  $\beta c$ :

$$n_{\rm m} = 2.2 \ (\beta \gamma)^{-2} \eta^{-1.54} \ {\rm m}^{-2}$$
 (5)

This relation is shown graphically in Fig. 2.

If the absence of the counter-jet is due to Doppler beaming, then the speed required would be  $\beta > 0.7$  ( $\gamma > 1.4$ ), and the ambient density  $n_{\rm m} < 0.6~{\rm m}^{-3}$ . Such a low density is implausible because: (1) it is even lower than the universal density, estimated to be 10% of the closure density from quasar statistics<sup>16</sup>; (2) 3C273 is a member of a group of galaxies<sup>17</sup> where the expected density would be intermediate between the universal value and the value ( $\sim 10^{+3}~{\rm m}^{-3}$ ) found in large clusters<sup>18</sup>; (3) the formation of a group of galaxies and a massive quasar presents difficulties in a region with a very low density; (4) the sideways confinement of the jet requires a density several orders of magnitude higher than  $0.6~{\rm m}^{-3}$ .

The ram pressure argument may be used in reverse. If  $n_{\rm m}$  lies between the closure density ( $\approx 20~{\rm m}^{-3}$ ) and the upper limit of  $\sim 5,000~{\rm m}^{-3}$  set by X-ray observations (R. Willingale, personal communication), then the forward speed is in the range  $0.02 < \beta < 0.3$ . This value refers to the speed of the bow-shock in front of the head. In conventional radio source models much of the emission is from a 'working surface' moving at a speed close to that of the bow-shock. If this is the case in 3C273, a receding counter-jet with  $\beta < 0.3$  would be detectable well above our sensitivity limit, and we conclude that the ejection must be to one side only.

A source model with one-sided ejection (model A) still has unexplained features. Unless the temperature of the ambient gas exceeds 108 K, hydrostatic gas pressure will not confine the jet sideways, and some other mechanism must be cited to

Table 1 Observed parameters of 3C273

Projected separation between nucleus and head of jet 40 kpc Size of head 2.1 kpc  $\times$  1.1 kpc Apparent opening angle of jet 0.03 rad Spectral index  $\alpha(S \propto \nu^{-\alpha})$  0.7 Depolarization wavelength  $\lambda^{1/2}$  of head 0.7 Brightness temperature of head 2.4  $\times$  108 K

achieve confinement. A further consequence of model A comes from the following statistical argument (see Fig. 3 and ref. 4). If one-sided sources commonly consist of relativistically moving cores (emitting radiation beamed into a solid angle  $\sim \gamma^{-}$ together with slow-moving outer lobes emitting unbeamed radiation, then for every 'core-dominated' source, there should be  $\sim \gamma^2$  sources with a single lobe and little or no core. The total number of one-sided core-dominated sources exceeds 50 (I. W. A. Browne, personal communication) but no single example has yet been found of a one-sided source with no core. Hence, the motion in the core of a one-sided source cannot be at an appreciably higher  $\gamma$  than the motion in the outer lobe. If this argument is accepted, the superluminal velocity observed in the core of 3C273 cannot be interpreted as a ballistic motion, and must instead be a light-echo or similar effect.

We may avoid these conclusions if we assume that in 3C273 the radio radiation is not emitted from slow-moving regions such as the 'working surface', but instead is emitted directly from a fast beam, such as that proposed in the beam-model by Blandford and Rees<sup>5</sup>. We have explored a model along these lines (model B) in which the ejection is relativistic and may (but need not) be two-sided. The superluminal proper motion in the nucleus corresponds to true ballistic motion with  $\gamma \sim 5$ , and this velocity persists unchanged out to distances of 200 kpc (20 arc s) from the nucleus. Because of the high Doppler beaming factor  $(\sim 100)$  the radio emission from the fast beam will dominate over that from slow-moving material. However, the absence of a detectable counter-jet implies that the rest-frame luminosity of the fast beam is at least as great as that of the working surface, which would seem unlikely in view of the greatly increased turbulence at the working surface<sup>5</sup>. This difficulty is avoided if the ejection is one-sided even in model B.

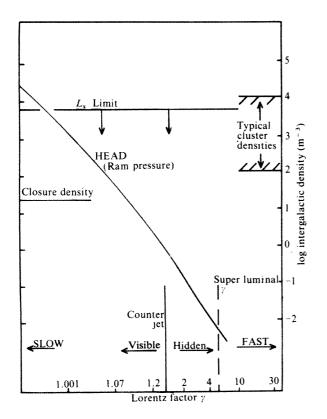


Fig. 2 The density of the intergalactic medium which will confine the head of 3C273 by ram-pressure as a function of forward speed, expressed as the Lorentz factor,  $\gamma$ , of the head. The actual function used is  $n_{\rm m} = 2.2 \ \beta^{-2} \gamma^{-2} \eta^{-1.54} \ {\rm m}^{-3}$ . The  $L_{\rm X}$  limit is provided by unpublished results from the Einstein satellite (R. Willingale, personal communication) and assumes a temperature of 108 K for the intergalactic medium. The speed at which an intrinsically equal counter-jet will be hidden by the Doppler effect is indicated, as is the value corresponding to the superluminal expansion

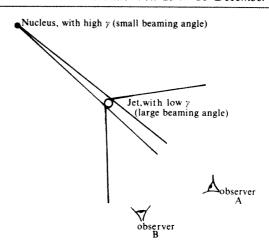


Fig. 3 Model for a radio source with a high  $\gamma$  in the nucleus and a low  $\gamma$  in the jet. Observer A sees strong radiation from both nucleus and jet. However a more typical observer B would see strong radiation from the jet but little radiation from the nucleus.

If the radio emission is from the fast beam directly, the beam must consist of relativistic particles, rather than photons. The thermal matter responsible for Faraday depolarization must not be co-moving, and hence cannot lie within the jet. It must form an irregular foreground screen, very possibly in the 'cocoon' region surrounding the beam. The front bow-shock moves slowly, and the ram pressure condition may be satisfied by the same values as in model A  $(0.02 < \beta < 0.3, 5000 > n_m > 20 \text{ m}^{-3})$ . There may be a difficulty with the power requirements in model B: to supply the fast-moving beam continuously requires at least  $5 \times 10^{38}$  W, which is 1% of the power output of the central quasar<sup>19</sup> and 100 times the emitted radio power.

The difficulties associated with these energy problems which are not unique to 3C273 have been discussed by Rees<sup>3</sup>. In the case of 3C273 the power flowing down the jet in the form of random particle motions is at least a factor  $100^{4/7}$  greater at the head than at the innermost end of the jet, which suggests that (in model B) the jet of 3C273 is not a continuous flow system.

We conclude that if the jet of 3C273 corresponds to the radio lobe of a normal radio source, then the emission is virtually unbeamed, and the ejection of material from the nucleus must be to one side only. Alternatively, the jet may be a visible example of a fast beam, such as that proposed in the beam model<sup>5</sup>, in which case the ejection may be two-sided, as Doppler beaming will suffice to hide the receding counterpart. Other factors have to be invoked, however, to explain the absence of unbeamed radiation from the radio lobe which such a fast beam would normally produce.

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#### Halo around the Crab Nebula

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It has been argued that the supernova of AD 1054 which left the Crab Nebula supernova remnant was of Type II. Such supernovae occur in the spiral arms of galaxies and are believed to have initial masses of  $>4M_{\odot}$ . The helium overabundance detected in the Crab Nebula filaments is compatible with an initial stellar core mass of  $2-4\,M_{\odot}$  corresponding to a total precursor mass of  $10-15\,M_{\odot}$ . The Crab Nebula as usually depicted consists of filaments of total mass  $2-3\,M_{\odot}$  and a pulsar of  $1.4\,M_{\odot}$ . If the precursor of SN1054 was heavier than  $\sim 4\,M_{\odot}$ , its outer envelope could be detectable as an extended halo around the Crab Nebula. We have detected such an outer halo, whose size implies that the progenitor of SN1054 was indeed a massive star.

The observational material was a deep IIIaF plate obtained on 28 December 1976 from the 1.2-m UK Schmidt Telescope specifically to search for a halo around the Crab, and partly analysed but displaced from our attention by other interesting observations in June 1978. The exposure was through an interference filter with a 150 Å bandpass centred on  $H\alpha$ , and was calibrated with a step wedge illuminated with broadband red light. The calibration curve had to be extrapolated from densities  $\sim 0.3$  to the sky density  $\sim 0.2$ , very near the toe of the curve, and our estimate of the intensity of the halo must be treated with caution. An area  $40 \times 40$  times arc min around the nebula was scanned on the PDS microdensitometer at the Anglo-Australian Observatory and calibrated by the step wedge exposed on the plate. The point-spread-function (p.s.f.) of the instrumentation is shown by images of stars and has a circular disk ~1.5 mm (1.7 arc min) in diameter (caused by multiple reflections). Several star images showing this disk arising from a central density comparable with that deep within the nebula were also scanned in the PDS machine to determine the p.s.f. beyond the disk. It drops to zero (<1% of night sky brightness) at 2 arc min radius from the stars.

The elliptical image of the Crab Nebula has a bright central area of 7 arc min major axis by 4 arc min minor axis, in accord with the dimensions derived by van den Bergh<sup>3</sup>. Beyond this abrupt boundary is a halo whose brightness falls to the night sky background at a distance from the centre of the nebula of 6 arc min along the minor axis direction (Fig. 1) and 14 arc min along the major axis direction, well beyond the tail of the p.s.f. The dimensions of the halo are thus three to four times that of the nebula.

The surface brightness of the halo is  $\sim 2\%$  of the night sky intensity. At Siding Spring, Bessell<sup>4</sup> measured the *R*-band sky brightness at 21.9 mag arc s<sup>-2</sup>. The night sky brightness is a variable reference, depending on season and solar cycle, but through the filter the Crab halo brightness is  $\sim 2 \times 10^{-7}$  erg s<sup>-1</sup> cm<sup>-2</sup> sr<sup>-1</sup>. This corresponds<sup>5</sup> to an emission measure for the H $\alpha$  intensity of  $\sim 7$  pc cm<sup>-6</sup>, with an uncertainty of a factor of two, corresponding to errors in the plate calibration and reference source.

A thermal soft X-ray enhancement peripheral to the nebula has been detected<sup>6</sup> from a lunar occultation observation of the Crab. Imaging soft X-ray observations from the Einstein Observatory have failed to confirm the reality of the halo because of the problems of scattering of the intense nebular

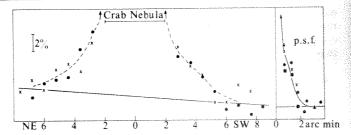


Fig. 1 Two surface brightness profiles (● and ×) along the minor axis of the Crab Nebula. The profiles are from parallel strips 1.6 arc min wide lying either side of the minor axis. The Crab Nebula has a bright central area 4.0 arc min in minor axis, with a halo extending 4 arc min beyond this. Also shown is the point-spread function of the instrument and measuring technique. The night-sky background under the Crab Nebula shows a slope up to the Milky Way in the north-east.

emission from the telescope mirror; modelling of this scattering may yet reveal a residual halo (F. Seward, personal communication). An optical halo extending 20% further from the centre than the abrupt edge of the filaments has been detected by Scargle<sup>7</sup> in the continuum bandpass 530–590 nm, and attributed to leaking electrons and magnetic fields, yielding extended synchrotron emission. As the halo we have detected through the  $H\alpha$  filter extends further and appears to be stronger we attribute it to  $H\alpha$  emission, and Scargle's halo we suppose to be weak line emission.

The origins of nebula and halo are proposed to be the following<sup>1</sup>. The core collapse deposited  $\sim 2 \times 10^{51}$  erg in the helium-rich outer core, which, expanding outwards, was decelerated, thus setting up a high-pressure region. consequent reverse shock wave drove the core material back to the centre. As the original deceleration was Rayleigh-Taylor unstable, some mixing of outer-core and envelope is expectedonly  $0.5 M_{\odot}$  of mixed envelope material is required to provide the observed nebula abundances1. The bulk of the supernova energy was transferred to the outer envelope of the presupernova star. On this model the observed nebula is the outer core-envelope mixture, whose present kinetic energy has been derived almost entirely from the pulsar, and the postulated halo is high-velocity low-density material from the envelope of the precursor. A consequence of such an interpretation is that the near coincidence between the supernova in history and the proper motion age for the nebula is merely coincidence.

An alternative explanation for the halo is that it represents light scattered off dust grains surrounding the Crab. These may have been produced by the precursor (an alternative indication that it was massive) or be swept-up interstellar material (J. C. Wheeler, personal communication) even at the low ambient interstellar density near the Crab ( $z \sim 200$  pc above the galactic plane).

The size and brightness of the observed optical halo compare well with the properties of the outer shell of the Crab predicted by Chevalier<sup>1</sup>. He calculated that a shell moving at  $5,000~\rm km~s^{-1}$  would have a size R four times the radius of the Crab. We observe a halo 3-4 times the Crab radius. If the shell was of mass  $M=10~M_{\odot}$ , mean density 1 atom cm<sup>-3</sup>, it would be fully ionized by the Crab's synchrotron radiation and have an emission measure of 5 pc cm<sup>-6</sup>. We observe  $E\sim7$  pc cm<sup>-6</sup>. Thus the halo we observe may be slightly more compact and brighter than the prediction. The mass in the shell is most sensitive to the extent of the halo: M scales as  $E^{1/2}R^{5/2}$ , yielding  $M\sim8M_{\odot}$ , so Chevalier's prediction is satisfactory. Adding filaments and pulsar, the precursor mass was about  $12~M_{\odot}$ .

The above calculation is based on the hypothesis of a uniform shell density. M is overestimated if the shell is lumpy or structured. The lack of granularity and the lack of peripheral brightening in the shell we observe are surprising but a massive progenitor would be expected to experience significant massloss before it went supernova and a substantial density gradient surrounding the expanding envelope might explain the observed

brightness distribution. In view of uncertainties in the model, in the reference intensity of the night sky and in the calibration of the plate, our estimate of the Crab precursor's mass of  $12 M_{\odot}$  is indicative only.

Observations of the halo, such as photoelectric confirmation of its extent, distribution and brightness, spectral confirmation of its emission spectrum and detection of its anticipated large velocity dispersion, and polarization measurements are needed to elucidate its nature.

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### Spin directions of interfering beams in quantum interferometry

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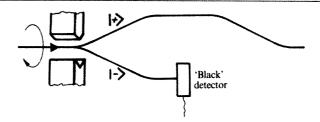
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It is shown here that, in a fermion interferometer experiment, the notion of the relative orientation of the interfering beams can be given a sensible meaning—information may be extracted about the spin directions without destroying the interference pattern. This is done on a gedanken-experiment level by introducing into, say, a neutron interferometer Stern-Gerlach magnets with detectors placed into their respective 'down'-spin beam paths. Similar considerations apply to experiments where spinor behaviour has been demonstrated in systems with higher spin.

In interferometry experiments with non-zero spin particles interesting effects arise due to the wave function (or probability amplitude) of the interfering beams being nonscalar. One effect concerns the sign change of a spinor wave function under  $2\pi$ -rotations<sup>1,2</sup>. This was verified by neutron interferometry experiments<sup>3-7</sup>, in which an incident beam was split coherently into two wave trains one of which was passed through a static magnetic field where it experienced Larmor precession and hence spin rotation. The spinor property was then found as a variation of the intensity of the recombined beams with a fringe period of multiples of  $4\pi$  of the rotation angles. The values of the rotation angles were calculated from the magnetic field strengths using the known value of the neutron magnetic moment.

The interpretation of this type of experiment has been widely debated<sup>8-12</sup>. Moore<sup>8</sup>, in discussing Bernstein's proposal<sup>2</sup> to use Larmor precession to implement the desired rotation, claims that in this experiment it would not be possible to observe directly that anything is actually rotating.

Byrne<sup>9</sup> has explicitly demonstrated the differences between spinor and vector wave interferometry. He showed that, in a spinor experiment, it is not possible to observe simultaneously the interference pattern and to obtain information about the relative spin directions of the constituent beams from measurements on the recombined beam. Byrne concludes that the notion of relative rotation ceases to have a meaning as it corresponds to nothing which is measurable. Thus he dismisses the interpretation that the experiments have provided a direct observation of the sign reversal of a spinor wave function subjected to a  $2\pi$  rotation as unsatisfactory because, for any



Modified Stern-Gerlach arrangement with a black detector in one beam path.

fermion the relative rotation of the spins in the two beams and the interference pattern are mutually incompatible observables.

However, it is possible to conceive of experimental schemes where information may be extracted from the beams interfering about their spin directions without destroying the interference pattern. In our experiments unpolarized incident neutrons were used for intensity reasons only. We consider here polarized incident neutrons that are able to trace the behaviour of the interfering spin states.

We look for a measurement procedure which changes neither the amplitudes nor the relative phase of these beams—a measurement without wave packet reduction. In quantum mechanics, such a measurement is possible if the quantum system under investigation is in an eigenstate of the observable defined by the measuring apparatus. For spin measurement, we consider for example, a Stern-Gerlach experimental arrangement in which we place into one of its separated beam paths a black (100% absorbing) detector (Fig. 1). To restore the beams to their initial paths, we place behind the first Stern-Gerlach an inverted second one. If the incoming beam is in the eigenstate corresponding to the detector-free path no event will be registered in the detector. The restriction that the amplitude at the detector never vanishes exactly due to the nonlocality of wave packets, can be reduced by increasing the separation of the beams. Similar considerations apply to the non-existence of exactly 100% absorbing detectors. Thus, the result that we did not register events in the detector together with the knowledge that particles have passed through the Stern-Gerlach gives us information about the spin observable of the incident beam.

In the more general case where a spin rotation device is placed in front of the Stern-Gerlach, the particles are no longer in an eigenstate of the Stern-Gerlach. Therefore, events will be registered in the detector. For simplicity we assume the incident beam to be polarized in a direction normal to its propagation direction and the magnetic field to rotate the spin around that propagation direction. The spin direction of the beam can then be found by rotating the Stern-Gerlach around the neutron propagation direction to that angular position where the counting rate in the detector vanishes. This spin direction is a macroscopically observable quantity—the angular position of the Stern-Gerlach. Many other geometrical arrangements can be envisaged on a gedanken-experiment level because for slow particles such as thermal neutrons the spin can be made to point in any direction relative to the propagation direction.

Formally we may describe our arrangement by a projection operator of the form  $P = |s\rangle\langle s|$ , where  $|s\rangle$  is the +eigenstate of the Stern-Gerlach. If that operator acts on the incident state |s>, that state is reproduced.

In an interferometer experiment of the type shown in Fig. 2 an incoming wave is split into two partial waves by a semireflecting mirror. These waves follow two distinct paths within the interferometer. Furthermore, in one beam path a magnetic field is arranged to rotate the spins. To observe the spin directions of these interfering beams we place a modified Stern-Gerlach arrangement into each interferometer beam path. In both arrangements we again place a detector into one of its beam paths, say, the 'down' spin beam path, leaving the 'up' beam path through each Stern-Gerlach free. If we start the experiment with arbitrary angular settings of the analysing direction of the Stern-Gerlach magnets we will again register particles in

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the detectors. The count rate in each detector will be lower in the interference experiment because the amplitude of each separated interferometer beam is lower than the amplitude of the incoming wave. But the fact that particles are registered means that the corresponding partial wave is not in the 'up' eigenstate of the Stern-Gerlach in that partial beam.

Both Stern-Gerlachs are now rotated independently of each other until we arrive at a position where neither detector registers a single event. At that point, the amplitude of the corresponding partial wave in the 'down' eigenstate of the Stern-Gerlach vanishes. However, we can still observe the interference pattern of the recombined beams, because our Stern-Gerlach arrangement does not enable us to determine which path is followed by the particle in the interferometer. Hence, both the null-effect spin measurements and the interference fringe observation allow us to conclude that the particles are in a coherent superposition of the two 'up' states defined by the two different Stern-Gerlachs. Or  $|\psi\rangle = |S_1\rangle + |S_2\rangle$ , where  $|S_1\rangle$  and  $|S_2\rangle$  are the 'up' eigenstates of the Stern-Gerlachs in the beam paths 1 and 2, respectively.

As the analysing directions of the Stern-Gerlachs are macroscopically observable quantities we conclude that the relative spin directions of the interfering beams are operationally meaningful quantities. This also applies to the relative rotation of the interfering beams because, in principle, we can trace the effect of the spin rotation device in arbitrarily small steps. We still have to consider whether the introduction of our spin-measurement devices may destroy the relative phase of the interfering beams.

Nevertheless, when considering—on the gedanken-experiment level—the use of the Stern-Gerlach magnets the inhomogeneous magnetic fields may lead to a destruction of the coherence of the interfering beams<sup>13</sup>. But other spin measurements can be envisaged where this is not the case. For thermal neutrons, diffraction at perfect crystals in external homogeneous magnetic fields<sup>14</sup> and refraction at wedge-shaped magnetic fields<sup>15</sup> both lead to a separation of the spin states while retaining their coherence properties. These schemes also offer the advantage that in contrast to a gedanken-experiment they are actually realizable experimentally along the lines of the existing technology of perfect crystal neutron optics.

At about the same time that the neutron interferometer experiments were performed it was realized  $^{16}$  that the spinor behaviour can also be observed in a spin 1 system. This exploits the fact that the wave function of a two-state system—except for the photon and similar degenerate cases—changes sign under a  $2\pi$  rotation. Thus, if in a three-state system the populations of two levels are inverted twice by properly phased electromagnetic high frequency pulses, their state function acquires the -1 phase factor. That phase factor may be revealed by observing interferences between one or both of these two states and the third state. There is also experimental evidence  $^{16}$  for that

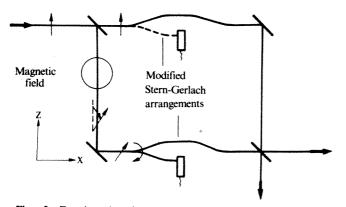


Fig. 2 Fermion interferometer gedanken-experiment with modified Stern-Gerlach arrangements in both beam paths. The magnetic field produces a spin rotation around the x-direction. A corresponding rotation of the lower Stern-Gerlach ensures unattenuated passage.

behaviour based on radio-frequency transitions between hyperfine energy levels in TIF. The same effect can be demonstrated experimentally by exploiting NMR transitions  $^{17-19}$ . There, systems can be found where a continuous transition between spin  $\frac{1}{2}$  and spin 1 behaviour may be seen depending on a continuous variation of the relative amplitudes of excitation of transitions between 2 or 3 levels.

Even for the above type of experiment we could consider a spin direction which is rotated while the relative population of two levels is changed. To apply our spin-measurement scheme we consider, again on a gedanken-experiment level, a further Stern-Gerlach magnet which separates spatially all three incoming states. Later while one of these states is left unaffected we may recombine the other two states and subject them to the same measurement procedure as above. Thus we may introduce transitions between these two states and we may use one of our modified Stern-Gerlach arrangements to determine the spatial direction for which this two-state subsystem is in an eigenstate. After recombination with the unaffected third state the interference effects may still be observed. Thus, here too it is possible to assign a meaning to the notion of a spatial direction, the rotation of which is associated with the  $4\pi$  periodicity of the phase factor.

The approach presented here is to some extent complementary to Renninger's considerations of a measurement without disturbance of the measured system<sup>20</sup>, a concept which has been extended to the spin case by Yoshihuku<sup>21</sup>. The difference is that in Renninger's case the wave packet is actually reduced by the measurement while we consider a measurement without wave packet reduction. Note that there exists a non-vanishing probability that in our modified Stern-Gerlach experiment we may not observe particles in the 'down' detector even if the spin is not in the 'up' eigenstate of the Stern-Gerlach. Evidently that probability can be reduced by increasing the number of particles passing through the apparatus and by carefully orienting the direction of the Stern-Gerlach. Therefore the spin direction can only be determined within a certain error. Our gedankenexperiment shares this this inherent statistical nature with other quantum measurements and, to some extent, with classical measurements. Nevertheless, in an interferometer experiment this is a minor point as our modified Stern-Gerlachs actually produce the beams in their respective 'up' eigenstates: the down' states are absorbed by the detectors.

The action of our modified Stern-Gerlach magnets including the absorbing detector in one beam path can be described by the projection operator

$$P_{\rm Op} = \frac{1}{2}(I + \mathbf{n}\sigma) \tag{1}$$

Here,  $\mathbf{n}$  is a unit vector pointing in the analysing direction of the Stern-Gerlach and  $\boldsymbol{\sigma}$  is the Pauli spin matrix vector. A state is in the 'up' eigenstate of the Stern-Gerlach if

$$\psi = P_{\mathrm{Op}}\psi \tag{2}$$

If we write  $\psi$  as the spinor

$$\psi = e^{i\xi} \left( \frac{a}{h e^{i\phi}} \right) \tag{3}$$

Equation (2) implies that

$$\mathbf{n} = (2ab\cos\phi, 2ab\sin\phi, a^2 - b^2) \tag{4}$$

that is **n** has to coincide with the polarization direction of the particles described by  $\psi$ .

Disregarding the details of the physics of the beam splitters we can use phenomenologically for the beams inside the interferometer the four-component spinor

$$\psi_{1F} = \begin{pmatrix} \psi_{1}^{+} \\ \psi_{1}^{-} \\ \psi_{2}^{+} \\ \psi_{2}^{-} \end{pmatrix} = e^{i\xi} \begin{pmatrix} a_{1} \\ b_{1} e^{i\phi_{1}} \\ a_{2} e^{i\chi} \\ b_{2} e^{i(\chi + \phi_{2})} \end{pmatrix}$$

where, say,  $\psi_1^+$  is the amplitude in the +z-direction in beam 1 and so on. The normalization condition is  $a_1^2 + b_1^2 + a_2^2 + b_2^2 = 1$ . This four-component spinor describes the states inside the

interferometer, the beams leaving the interferometer are superpositions of its various amplitudes.

In analogy with the above case, the projection operator describing the case where two modified Stern-Gerlachs are in the interferometer, one in each beam path, may be given as

$$P_{\rm IF} = \frac{1}{2} \begin{pmatrix} I + \mathbf{n}_1 \boldsymbol{\sigma} & 0 \\ 0 & I + \mathbf{n}_2 \boldsymbol{\sigma} \end{pmatrix} \tag{6}$$

The condition that this operator leaves the wave function invariant

$$\psi_{\text{IF}} = P_{\text{IF}} \psi_{\text{IF}} \tag{7}$$

implies again that

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$$\mathbf{n}_{i} = \frac{1}{a_{i}^{2} + b_{i}^{2}} (2a_{i}b_{i}\cos\phi_{i}, 2a_{i}b_{i}\sin\phi_{i}, a_{i}^{2} - b_{i}^{2})$$
(8

Or, equivalently, the analysing directions of the individual Stern-Gerlachs have to be oriented parallel to the respective beam polarization directions. The particle state is then left invariant, in particular the relative phase  $\chi$  in equation (5) between the two interferometer beams is unchanged. Therefore the interference pattern may still be observed.

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# **Detection of monsoon** inversion by TIROS-N satellite

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Colon<sup>1</sup> and Ramage<sup>2</sup> have investigated the thermal stratification of the summer monsoon air and presented evidence of a welldefined temperature inversion in the lower atmosphere over the Arabian Sea. This inversion is low (base between 900 and 800 mbar) and strong over the western Arabian Sea and weakens and rises (base at ~700 mbar) towards the coast of India and is not observed east of 70°E, especially during the active monsoon3. The presence of dry warm continental air from Africa and Arabia above the maritime air is thought to be associated with this inversion. This inversion is very important to the rain producing potential of the monsoon current because once the inversion is destroyed there is a favourable stratification for rapid release of moisture upwards leading to precipitation. Observations of the western Arabian Sea inversion features have previously been reported only from in situ ship radiosonde and aircraft dropsonde measurements. Although the basic accuracy and the vertical resolution of the present-day satellite sensors cannot delineate the small-scale variations of temperature4 such as monsoon inversions we have detected these features from just the TIROS-N derived seasurface temperatures and the 1,000-850 mbar layer-mean temperatures using a simple differencing procedure. From these temperatures and simultaneous satellite-derived mid-tropospheric water vapour content, we show here the close link between the extent of inversion regions and the convective processes with the Indian monsoon at its different phases.

The present data pertain to the Monex-period (1 May-31 July 1979) TIROS-N satellite results of 14.00 h LT supplied by NOAA Environmental Satellite Service of the USA. These results (about one set in a 2.5° × 2.5° lat.-long. box) include data on the: sea surface temperature (SST); 15 layer-mean atmospheric temperature profile from 1,000 to 0.4 mbar; and 3 level total water vapour content.

The inversions are characterized by the altitude of its base, height extent and temperature departure. Well-marked (height extent >30 mbar and temperature departure >3 °C) monsoon inversions in the western Arabian Sea exhibit in the aircraft dropsonde profiles a negative lapse rate in the altitude regions between 900 and 800 mbar region, above and below which the lapse rates are similar to those of the normal profiles.

Our investigation did not reveal any inversion features from the individual temperature profiles of the satellite data set. However, we now use only the SST and the lowest layer-mean (1,000-850 mbar) temperature, referred to as  $T_1$  and  $T_2$ respectively.

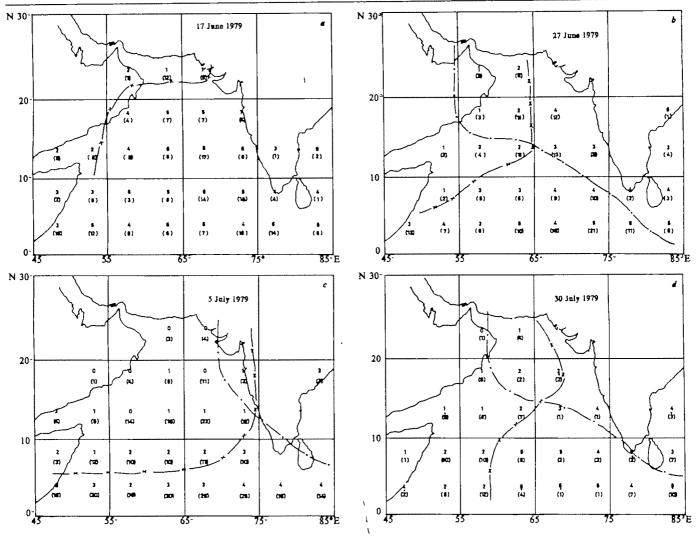
For a temperature profile with an inversion structure at lower levels (below 850 mbar), the layer-mean temperature of the 1,000-850 mbar layer will be larger than that for normal profiles (those which follow a standard lapse rate with height). Assuming further that the sensible heat exchange between the sea and the air above is small and fairly uniform<sup>5</sup> (implying small air-sea temperature difference), it is to be expected that a horizontal map of the difference,  $\Delta T$ , of the satellite-derived SST  $(T_1)$  and the 1.000-850 mbar layer-mean temperature  $(T_2)$ , with appropriate time and spatial averaging could reveal the inversion regions. Lower values of  $\Delta T$  can be interpreted as being associated with regions of inversion. From in situ aircraft dropsonde measurements, the values of  $\Delta T$  for non-inversion profiles in the active monsoon areas range between 4 and 6 °C and in inversions these are as low as 0 °C in some cases.

Examination of the horizontal variation of the  $\Delta T$  values thus obtained from satellite data also minimizes the errors that may be present in the original temperatures  $T_1$  and  $T_2$ , whose retrieval involves assumptions of the atmospheric models in the radiative transfer computations.

The aircraft dropsonde measurements during Monex 1979 provided data for in situ ground truth comparisons in the Arabian Sea between long. 75° E and 55° E. However, these measurements were available up to 27 June 1979 covering only the onset and active periods of the monsoon. No in situ data

Table 1 Comparison of aircraft profiles with satellite data

	Aircraft	Near simultaneous satellite data		
	profiles	$\Delta T \leq 2 ^{\circ}\text{C}$		
No. of profiles with well-marked inversion below 850 mbar	30	23	7 (for four of them $\Delta T = 3$ °C)	
No. of profiles without well-marked inversion	129	0	129	



; <del>\</del> ;

Fig. 1 a,  $\Delta T$  map of the onset phase (3-day average). Values in parentheses denote the number of observations available in the  $5^{\circ} \times 5^{\circ}$  box for 3 days. —×—, The boundary of the inversion region. b, As a for the active phase (3-day average). —·—, The boundary, to the east of which water vapour content in the 700-500 mbar layer is 10 mm or more and to the west less than 10 mm. c, As b for the weak phase (5-day average). d, As b for the revival phase (3-day average).

were available for break and revival periods. The *in situ* information available has been used to define inversion regions (up to 850 mbar) in the Arabian Sea.

A total of 85 and 74 aircraft profiles during the onset and active phases respectively were used for comparisons with the satellite data. A fairly large number (20) of well-marked inversions were available only from the profiles of 27 June. The comparative study indicates that inversion-free (up to 850 mbar) regions, as evidenced from dropsonde data, were associated with satellite  $\Delta T$  values of 3 °C or more, and well-marked inversion profiles were associated with  $\Delta T$  values of 2 °C or less.

Table 1 shows that with a high reliability the  $\Delta T$  values can be used to determine the existence or otherwise of the inversion regions. These comparisons (and the results) indicate that the  $\Delta T$  variations inferred from our method could be considered accurate to 1°C.

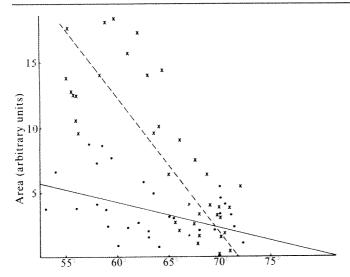
We also attempted to look for inversions above 850 mbar by employing the  $\Delta T$  maps derived from the 1,000–850 and the 850–700 mbar layer-mean temperature values. No significant horizontal variations of  $\Delta T$  were observed, indicating that these are either weak, less in height extent or non-existent. The detection of inversions in these regions would need much refined satellite measurements.

The *in situ* statistics of the aircraft dropsonde profiles were used to interpret all available results and  $\Delta T$  values of 2 °C or less have been delineated as regions associated with inversions.

The data at any particular location for any individual day were insufficient for our analysis, mainly due to a large underlap in the tropical regions of about  $8-10^\circ$  in latitude between successive satellite passes, the swath itself being  $\sim 10^\circ$  lat., the other major limitation was the cloud cover which inhibited proper temperature retrievals. To help overcome these limitations we adopted a spatial (a  $5^\circ \times 5^\circ$  lat.-long, grid box) and time-averaging procedure to investigate the inversion regions in the temperature fields. These optimal nesting and averaging schemes were arrived at from a preliminary examination of the day-to-day plot of the satellite temperature maps and a consideration of the large time and spatial scales associated with the monsoon inversions.

Figure 1 presents the  $\Delta T$  maps during the different phases of the monsoon of 1979. Values of  $\Delta T$  indicated over land correspond to the  $5^{\circ}\times 5^{\circ}$  box central location. The contribution is due entirely to those soundings from over the sea. A feature which persists throughout the observational period is the tendency of  $\Delta T$  values to increase slowly from west to east as the monsoon becomes established.

The map of the pre-onset time (not reproduced here) shows  $\Delta T$  values at all places more than 3 °C indicating no inversion features. Figure 1a shows the inversion being set up in the western Arabian Sea at the onset time of the monsoon. The low  $\Delta T$  values start covering a larger part of the Arabian Sea (but confined still to west of 65° E) at the most active phase of the monsoon (Fig. 1b). These results agree with those reported by



Strength of inversion at onset and active phases from in situ –, Onset phase (●); – – –, active phase (×).

Sen and Das<sup>6</sup> and Ghosh et al.<sup>3</sup>, who have shown from limited Monex (1979) and Ismex (1973) in situ data respectively, that the inversion regions are confined to west of 65° E during the active periods.

The area included between the normal profile (with standard lapse rate) and the observed profile (with or without inversion) is a good in situ measure of the inversion strength, as it incorporates both the height extent and temperature departure features of inversion. Figure 2 shows a scatter plot of the area so calculated between surface and 700 mbar for the in situ dropsonde profiles for the onset and active periods. The gradual increase of area from east to west and a sharp increase of the slope from the onset to the active phase as revealed by in situ observations of this plot agree well with similar features revealed by the satellite  $\Delta T$  maps (Fig. 1a, b).

We also report here observations of the inversion features available from the  $\Delta T$  maps during the weak and the revival phases of the monsoonal activity. No observations in situ or otherwise have previously been reported of the inversion features during these periods of the monsoon. Our analysis shows that during times of weak monsoon activity the inversion regions intrude east of 65° E (Fig. 1c) and even encroach the west coast of India. The revival of monsoon after 29 July is revealed by the  $\Delta T$  map (Fig. 1d), with a shifting to the west of  $\Delta T$  values, and these being 3 °C or more east of 65° E.

Figure 1b-d also shows the 10-mm total water vapour content contour corresponding to the 700-500 mbar level drawn from the data of the TIROS-N observations. East of this contour the values are higher and to the west lower than 10 mm. Figure 1b-d reveals a clear oscillation from west to east and then back to west longitudes of this contour between the active, weak and revival phases of the monsoon.

We have not observed such clear oscillations of the lower level (1,000-700 mbar) water vapour content contours. The observation of the middle level moisture content and its change during the different phases of the monsoon (illustrating the convective activity) are consistent with our observations of the east-west shifts of the low level inversions, and show the close link of these two phenomena with the general monsoon circulation.

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### Steady-state magma discharge at Etna 1971–81

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Throughout the past decade Mount Etna has been in almost continuous activity and even during periods of repose incandescent lava has often been visible in at least one of the summit vents. Using observations by Italian, British and French volcanological teams we have estimated the volumes of lava produced by each eruption from 1971 to July 1981. The computed output of magma for this period approximates to a rate of 0.7 m<sup>3</sup> s<sup>-1</sup>. We now compare this with our output rate estimates for Etna's historic past. The steady-state nature of the output during the past decade has implications for the interpretation of the volcano's internal plumbing and the petrology of its lavas, and the assumption that this state will be maintained allows us to discuss the timing and magnitude of future erup-

In 1975 we presented evidence<sup>1</sup> for the rate at which magma appears at the summit and at the flank vents of Etna during the period 1966-74 and for the rate at which it erupted solely on the flanks of the volcano over a longer period (1535-1974). We now extend this record of volumetric discharge of magma to July 1981 (Table 1).

During the period 1971-July 1981 there were six eruptions involving flank vents (1971, 1974, August 1978, November 1978, 1979 and 1981). The rest of the activity was at the summit vents (mainly north-east and south-east craters) and at secondary vents lower down the volcano (mainly to the north), some of which were fed by lava tubes from the summit vents. Many of the eruptions were short, explosive, high effusion rate events such as those in the latter half of 1977. Other eruptive episodes involved continuous effusion for months at a time<sup>2</sup> such as those typifying the period September 1974 to June 1976. Our volume data (Table 1) come from various sources and are not corrected for vesicularity. The most accurate of these data are the result of theodolite surveying of the summit region<sup>3-5</sup>. Most lava flow volumes lower on the flanks have been estimated from their areas and average thicknesses. The accuracy of these less precise estimates is probably better than  $\pm 50\%$ .

The cumulative volume plot for January 1971-July 1981 (Fig. 1) has a linear trend which gives an output rate<sup>6</sup> of 0.7 m<sup>3</sup> s (0.67 m<sup>3</sup> s<sup>-1</sup>). For the period September 1974-June 1976 when effusion was almost continuous, though variable over short periods of time, the rate was indistinguishable (0.67 m<sup>3</sup> s<sup>-1</sup>) from the overall 1971-July 1981 rate. After a period of repose during 1972-73 following the 1971 flank eruption the cumulative volume curve has kept within the limits shown in Fig. 1, which represents a range of volume of about 25×106m3. There is a tendency for a change in style of eruption during the second half of this steady-state period (July 1977-July 1981), coupled with a slightly increased gradient of the curve, but this appears to be of secondary importance.

We interpret these features of Fig. 1 as follows. From 1971 to July 1981 magma has been entering and leaving Etna at a rate of 0.7 m<sup>3</sup> s<sup>-1</sup>. At times this steady state has been achieved exactly (within the precision of our data) as in 1975 and is equivalent to the behaviour of Kilauea in 1952, 1967-68 and 1969-71 (see ref. 7). At other times magma is either stored in the volcano before release or the magma arrives in discrete batches which

Table 1 Volumes of the eruptions of Etna 1971-July 1981

	Eruption	Volume (m <sup>3</sup> ×10 <sup>6</sup> )	$\Sigma$ Volume $(m^3 \times 10^6)$	Ref.		Eruption	Volume $(m^3 \times 10^6)$	$\Sigma$ Volume $(m^3 \times 10^6)$	Ref.
1971	5 April-12 June	50	50	1	1977	10-13 December	0.5	129	
1971	June-September	7	57	1	1977	18 December	1	130	
1973	October	10	67	1	1977	24-25 December	1	131	
1974	31 January-17 March	6	73	20	1977	29 December	1	132 >	4, 24-27
1974	29 September-24 February	7	80	3	1978	2-4 January	2.5	134.5	
1975	24 February-12 September	13	93	2, 21, 22	1978	5 January	0.5	135	
1975	12 September-28 November	5	98	3, 23	1978	7 January	0.5	135.5	
1975	29 November-17 June	12	110	21, 23	1978	25-28 March	3	138.5	28
1976	20 August-8 January	6	116	21, 24, 25	1978	29 April-5 June	23	161.5	4, 24
1977	16-22 July	1	117		1978	23-30 August	3	164.5	4, 24, 29
1977	5-6 August	1	118		1978	23-30 November	5	169.5	4, 24, 29
1977	14 August	4	122		1979	3-9 August	12	181.5	24, 30
1977	24 August	0.5	122.5		1980	April	4	185.5	31, 32
1977	2-4 November	1	123.5	4, 24-27	1980	1 September	7	192.5	33
1977	7–8 November	1	124.5		1980	6 September	3	195.5	33, 34
1977	22 November	1	125.5		1981	5-7 February	4	199.5	35
1977	25 November	1	126.5		1981	17-20 March	20	219.5	36
1977	6 December	2	128.5						

All volumes estimated to the nearest  $1 \times 10^6 \,\mathrm{m}^3$  except eruptions from 16 July 1977 to 7 January 1978 which are estimated to the nearest  $0.5 \times 10^6 \,\mathrm{m}^3$ .

results in a punctuated pattern of magma release. The maximum capacity of either of these processes (or a combination of them) is  $\sim 25 \times 10^6$  m<sup>3</sup>.

Recognition of this rate of magma discharge and the capacity of the episodic process(es) since 1974 (Fig. 1) allows us to suggest how Etna will behave in the immediate future. If the cumulative volume curve is to remain within the empirically defined limits Etna must erupt again before about May 1982. At that time up to  $25 \times 10^6$  m³ of magma could be expected, but an eruption volume of less than about  $17 \times 10^6$  m³ could be expected from an eruption in January 1982 (Fig. 1). These are not predictions of specific events but general forecasts of the timing and magnitude of future eruptions based on the behaviour of

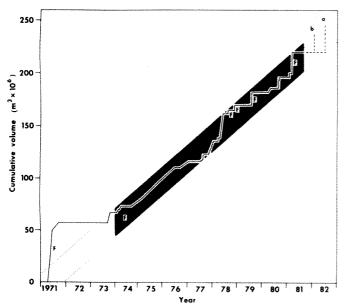


Fig. 1 Plot of the cumulative volume of magma erupted at Etna from 1971 to July 1981 listed in Table 1. F indicates eruptions involving flank vents. From January 1974 to July 1981 the cumulative volume curve has remained within the limits shown by the shaded area which corresponds to the average discharge rate calculated for the entire period 1971–July 1981  $(0.7 \text{ m}^3 \text{ s}^{-1})$ . If the volcano continues to behave in this way an eruption is expected to occur before approximately May  $1982 \pm 3$  months. An eruption at that time should not exceed a volume of about  $25 \pm 6 \times 10^6 \text{ m}^3$  (a). The maximum volume of an eruption in, say, January 1982 would be about  $17 \times 10^6 \text{ m}^3$  (b). If instead there is a larger volume eruption (as in 1971) a correspondingly long period of repose is expected to follow.

the volcano during the past seven years. The accuracy of such forecasts depends on the error involved in the volume estimates used in constructing the curve. Random errors in individual estimates (probably a few 10%) will tend to cancel in the cumulative volume curve and reduce the uncertainty in the limits of the shaded envelope of Fig. 1. Unknown systematic errors will affect the gradient of the curve. The above forecast depends in particular on the accuracy of the last eruption volume of the curve (March 1981), which is about  $\pm 10\%$  ( $20\pm 2\times 10^6\,\mathrm{m}^3$ ). Combined with an assumed uncertainty of the steady-state limits (shaded envelope) of  $\pm 20\%$  this produces an uncertainty of about  $\pm 3$  months and about  $\pm 6\times 10^6\,\mathrm{m}^3$  in the maximum repose period and volume of the next eruption.

The 1971 eruption does not fit the above pattern. From January 1966 until April 1971 summit effusion was very similar in style and magnitude to the 1975 activity8,9 and the rate of 0.7 m<sup>3</sup> s<sup>-1</sup> is also probably applicable to the 1966-71 period. If this is true then the 1971 eruption represents an event which involved an unusually large volume of magma and after which there was an unusually long repose period. The proposed steady-state rate of discharge from 1966 to 1981 of 0.7 m<sup>3</sup> s<sup>-1</sup> is equated with the rate of supply of magma from the lower crust and the 1971 eruption volume is part of that supply rather than originating in some additional reservoir or source region. The large volume implies that either a high-level reservoir was drained or that an unusually large batch of magma rose into the volcano, but that both were part of a steady supply system which required a subsequent period of recharge during 1972-73. If our assumption about the steady-state output rate from 1966 to April 1971 is incorrect then this weakens, but does not invalidate, the case for the 1971 eruption magma being part of the steady-state supply.

These conclusions reinforce the previously noted discrepancy between the modern discharge rate and the rate calculated from the flank eruptions of the past 200 yr (0.17 m³ s<sup>-1</sup>). We suggested that summit eruptions, whose volumes cannot be estimated for this period, might increase this latter value to 0.26 m³ s<sup>-1</sup>. However, the modern (1971–81) ratio of summit to flank magma volume of 56:44, if applied to the record of the past 200 yr, gives a combined summit and flank output rate of 0.39 m³s<sup>-1</sup>. There has been one historic period (1610–69) during which magma erupted at a rate (0.83 m³s<sup>-1</sup>) comparable with the 1971–81 rate¹. Between 1610 and 1669 there was no recorded summit effusion and the lavas of the very long-lasting flank eruptions were characterized by particularly large plagioclase phenocrysts 10. The large phenocrysts may imply longer residence times for magma at high levels than at present. Normal access to the summit vents may have been

blocked at this time and all the magma from depth may have found its way to the surface through flank vents.

The steady-state discharge of magma at Etna provides a framework within which some of the findings of recent research on the volcano can be correlated. The major element geochemistry of historical Etna lavas has shown little variation with time 10,11. A steady-state discharge rate must be an important factor in determining the chemical homogeneity of these hawaiite lavas. Small variations in residence time at high levels in the feeding fissures are probably capable of producing the observed limited variations in chemistry. Direct monitoring of SO<sub>2</sub> by correlation spectrometry in the plume of Etna during June 1975<sup>12</sup>, August 1977<sup>13</sup> and July 1978<sup>14</sup> has revealed a characteristic discharge rate for this species varying between 1.000 and 5.000 tonnes per day depending on the level of activity. A value within this range may represent the steady rate of release of SO<sub>2</sub> from a hawaiite magma flux of 0.7 m<sup>3</sup> s<sup>-1</sup> which is  $\sim 0.6-3$  wt.% of the mass of erupted magma (assuming the specific gravity of the magma is 2,650 kg m<sup>-3</sup>). However, this range is much higher than the sulphur contents of most sulphidesaturated basaltic liquids (<0.25 wt% S)<sup>15</sup>. If the spectrometer values are accurate then the Etna magmas are either exceptionally rich in sulphur or a larger volume of magma may be degassing than appears at the surface at the steady-state rate.

Various techniques for monitoring the surface deformation of Etna have been since 1971, including precise levelling, electronic distance measurements and several types of tiltmeters<sup>16-19</sup>. The most substantial deformations were measured from 1971 to 1974 during the period of recovery from the 1971 eruption<sup>16</sup>. Subsequent measured deformation has been relatively minor<sup>10</sup>. This suggests that there is no elastic reservoir system within the volcano that is sensitive to the discharge of up to  $25 \times 10^6$  m<sup>3</sup> of magma within the steady-state limits of Fig. 1.

We thank the many scientists whose observations on Etna over the past decade made this work possible. J.E.G. acknowledges financial support from NERC. G.W. is a visiting scientist at the Lunar and Planetary Institute which is operated by the Universities Space Research Association under contract NASW-3389 with NASA.

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### Charnockite and granite formation and influx of CO<sub>2</sub> at Kabbaldurga

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The discovery, at Kabbaldurga quarry, Karnataka, south India 1-3, of patches of charnockite apparently in an arrested state of development has refocused attention on the mechanism of charnockite formation. In particular, the relative roles of CO2 and H<sub>2</sub>O during high grade metamorphism and charnockite development can be studied in such an area. During an influx of a CO2-rich volatile phase, H2O may be liberated as a result of the reduction in the state of hydration by the breakdown of hydrous minerals<sup>4</sup>. The P-T conditions in which this occurs are important because, with an increase in XH2O, conditions which may allow anatexis could be initiated in advance of such a CO2-rich phase. Indeed, Weaver<sup>5</sup> has suggested that the K-rich acid charnockites found at Pallavaram, Madras<sup>6</sup>, developed due to changes in volatile composition before the onset of charnockite conditions. The exposure at Kabbaldurga quarry is interpreted here as displaying evidence that such a process has taken place.

During recent field work in the Archaean (~2,700 Myr) gneiss terrain between Mysore and Bangalore, Karnataka, south India, the southern end of the Closepet Granite including the area of Kabbaldurga was examined. This granite is a substantial body with a linear extension northwards for some 200 km to just south of Bellary. The country rocks into which the granite has been emplaced are dominantly polyphase, grey Archaean gneisses-the Peninsular Gneiss complex. In this area the gneissic complex has been subjected to upper amphibolite and granulite facies conditions of metamorphism. The age of the Peninsular Gneiss complex precursors is not well known, but is at least 2,600 Myr (ref. 8), an age which reflects a metamorphic event. More recently, an Rb-Sr whole rock isochron age of 3,358 Myr has been obtained for part of the complex to the west of Bangalore9. Two homogeneous foliated granitoid masses, which also form a portion of the Peninsular Gneiss, at Chickmagalur and at Chitradurga, have given Rb-Sr ages around 3,080 Myr and 2,790 Myr respectively (S. Moorbath and P. N. Taylor, personal communication). These ages for components of the gneiss complex are consistent with that of 2,670 Myr (ref. 10), interpreted as a metamorphic age, which has been obtained from the charnockite and the gneisses at Kabbaldurga and 2,615 Myr for the general granulite facies event<sup>8</sup> of the area.

Among the studies of the Closepet Granite 7,11,12 the observations that most closely approach those made during the present investigation are those of Radhakrishna7, who differentiated the granite into four main varieties. Briefly, the present investigation suggests that the granite is itself a polyphase body and comprises several pinkish and/or greyish, coarse-grained, porphyrytic and non-porphyrytic granites (using the nomenclature of Streckeisen<sup>13</sup>) and various pegmatitic and aplitic phases. In some portions of the granite enclaves of the country rocks are present. In these areas it is evident that pink alkalifeldspar megacrysts present in the porphyrytic granites are not phenocrysts but are of replacive origin because the megacrysts are developed in and across the margins of some of the enclaves. Additionally in some areas, white alkali-feldspar megacrysts have been partially replaced by veins and patches of pink alkali-feldspar. This accords with the observations of Radhakrishna<sup>7</sup> and with the hypothesis<sup>11</sup> that metasomatism is involved in the production of the granite. There is frequently a distinct orientation of both types of megacrysts which is parallel to a variably developed foliation picked out by an alignment of biotite and to the northerly trend of the granite body. This feature suggests that the megacrysts have developed in response to late-stage K-rich fluids in a still active stress zone. This fluid could also be the source of the enrichment in Th, Rb and Pb as

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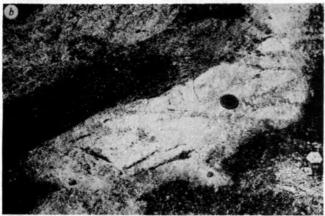


Fig. 1 The penecontemporaneous relationships of granite and charnockite. a, The nebulitic development of charnockite after the formation of granite. In the foreground and left-hand side grey migmatitic gneiss is cut by a sheet of granite. The right-hand contact of this sheet is gradational into the gneiss. Both of these lithologies have been overprinted by the nebulitic development of charnockite (see ref. 1) which forms the black areas. Running diagonally across the top right-hand corner of the photograph is an area of charnockite which occurs as anastamosing veins overprinting an area dominantly of granite. b, A thin sheet of granite ( $\sim$ 28 cm) without charnockite development, cutting and containing two schlieren of charnockite developed from grey gneiss. Above the granite sheet is a nebulitic contact between an area of charnockite and gneiss in which charnockite has begun to develop.

well as K reported from the northern portion of the granite body<sup>12</sup>. Additionally, the granite has a broad overall zonation<sup>7</sup> which is parallel to the northerly trend of the body.

On the southeastern side the rocks comprising the marginal contact zone of the granite are of particular interest. This contact zone consists of a series of intermixed, polyphase granitic sheets and veins (together with pegmatitic and aplitic rocks) enclosing and cutting enclaves of Peninsular Gneiss. In this area the Peninsular Gneiss consists of typical grey, banded gneisses with a few horizons of more basic material and some bodies of essentially unfoliated, more homogeneous gneiss. In the contact zone the enclaves display a range of textural and structural modification from relatively angular, unmodified gneisses into highly banded rocks passing into nebulitic and inhomogeneous, coarse-grained material which lacks a gneissic fabric and may be differentiated into neosome and palaeosome. Such material exhibits the structures and textures of migmatites 14,15. The transitions from these migmatitic rocks into more homogeneous granitic rocks are attributed to the process of anatexis and may be described as homogeneous and inhomogeneous diatexites (see ref. 15). From the field evidence in these areas I conclude that the origin of the Closepet Granite is due to partical melting in the Peninsular Gneisses. Crawford8 has determined the initial<sup>67</sup>Sr/<sup>86</sup>Sr ratio of the granite to be 0.705 ± 0.0014 which,

for  $\sim 2,400$  Myr, suggests a derivation by remobilization of pre-existing sialic material. The importance of this observation becomes apparent in the Kabbaldurga quarries where the relationships of the Closepet Granite to the formation of charnockitic rocks may be observed. The association of charnockitic rocks and migmatites is a feature which has long been known from other regions <sup>16</sup>. Kabbaldurga quarry is an area where data regarding this interesting relationship may be collected.

On close investigation the patchy development of charnockite can be seen to have occurred not only in the Peninsular Gneisses as has previously been described 1-3 but also in some of the rocks which form a part of the Closepet Granite. From field evidence the development of the charnockitic patches seems to be penecontemporaneous with the development of the granite. Veins and sheets of granite, without charnockitic assemblages, cut charnockitic patches in the gneisses yet are themselves cut by other granitic veins with charnockite development in them (Fig. 1). Note that the areas of gneiss in which chanockite has developed have not undergone partial melting3. However, in some of the grey, biotite gneisses around the patches of charnockite partial melting to produce granitic material has taken place. These rocks contain the structures and textures which closely resemble those found elesewhere along the southeastern marginal zone of the granite and which are interpreted as migmatitic and anatectic features. The onset of charnockite formation at Kabbaldurga is though to have taken place at between 3 and 5 kbar at 600-700 °C (ref. 3), (though this is a low estimate compared with the values suggested for the surrounding charnockite formation<sup>17</sup>, the mineral assemblages present do not allow a more accurate estimate to be made) and to have been initiated by an influx of a mantle-derived volatile phase rich in CO<sub>2</sub>. Such a mechanism has now been more widely applied to granulite facies rocks<sup>5,18</sup> to explain in particular the formation of hypersthene-bearing assemblages without a change in the existing P-T conditions. Whilst Janardhan et al. argue that a large rise of temperature is not required for the formation of charnockite at Kabbaldurga, the invading volatile phase may have brought a source of heat with it particularly as the volatile phase is considered to be mantle-derived3. Schuiling and Kreulen<sup>19</sup> attribute considerable potential to an invading volatile phase with respect to the release of heat and the initiation of anatexis. Although data enabling the application of their model calculations are not obtainable, any influx of heat will assist the breakdown of the hydrates present and the production of the observed migmatitic features. At Kabbaldurga, therefore, during such an advance of CO2, the hydrous minerals-in this case biotite and amphiblole-have broken down, without partial melting, to give hypersthene-bearing, non-foliated, charnockitic rocks. The H2O released by this breakdown would have immediately joined the volatile phase which would continue its upwards migration. Therefore, at any point with the continued breakdown of hydrous phases more H<sub>2</sub>O would be added to the volatile phase, eventually diluting the composition of the volatiles until such a time as the CO2 front advanced through that point. Although the invading volatile phase is K-rich3 there is no unequivocal evidence that this is a different volatile phase from that which gives rise to the growth of alkali feldspar megacrysts in the granite. I believe, however, that this latter phenomenon is a late-stage crystallization effect-in common with many other granites-and that the two volatile phases are not directly related.

The exposure at Kabbaldurga seems to have been in a rather special position, at the very front of the CO<sub>2</sub> advance, where the introduced volatile phases are subject to dilution by the released H<sub>2</sub>O. The H<sub>2</sub>O released by the breakdown of the hydrous phases has had serious consequences. In some areas of the grey gneisses not affected by charnockite formation, the granite solidus was reached and partial melting took place. Then, after the formation of the granite, the CO<sub>2</sub> front advanced into some of the areas where partial melt had accumulated and has formed K-rich charnockite. That there are areas of Closepet Granite in which charnockite has developed demonstrates that the two

events were penecontemporaneous. Weaver<sup>5</sup>, in discussing the generation of the K-rich charnockites at Pallavaram, considered that they represent an unusual component of an otherwise fairly typical Archaean grey gneiss-granulite terrain. He suggested that these K-rich rocks had developed due to a change of the fluid composition which allowed metasomatism and partial fusion to occur before the formation of charnockite by the advance of a CO<sub>2</sub> front. At the present level of erosion, the exposure is interpreted as having been well below the former position of the CO<sub>2</sub> front and all the rocks have been completley converted to charnockite.

At Kabbaldurga it appears that there is evidence not only of the arrested process of charnockite formation, but also of the process of crustal fusion induced by an accumulation of  $H_{2\mathrm{O}}$  in advance of an ascending CO2-rich volatile phase. Thus, this area of the Peninsular Gneiss complex and the Closepet Granite is fundamental to the understanding of the processes of both charnockite formation and granite formation deep in the crust.

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# Limitations on the scale of mantle heterogeneities under oceanic ridges

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The scale of mantle heterogeneities has been debated ever since the first observations of isotopic variations along mid-oceanic ridges<sup>1,2</sup>. Subsequent studies on lead and strontium isotopic variations along the Mid-Atlantic Ridge3-5 have shown that these isotopic compositions may vary with a major wavelength of  $\sim$ 100-1,000 km. The immediate question is that of the scale (if any?) at which one may consider a piece of suboceanic mantle under a ridge to be homogeneous. We have studied two segments of oceanic ridges at the scale of a few kilometres. One of these portions of oceanic ridge, the CYAMEX zone in East Pacific Rise is typical, with a strongly 'depleted' chemistry. The other one, with an 'intermediate' chemistry, is the FAMOUS zone of the Mid-Atlantic Ridge. Our results show isotopic homogeneity for both zones even though small Pb variations persist on a small scale.

The general geology of the FAMOUS area has been described elsewhere<sup>6,7</sup>. In the central valley of this slow spreading ridge (2 cm yr<sup>-1</sup>), we can distinguish two types of structures, real volcanoes such as Mount Venus, and lava flows, probably of fissural type. Several samples were collected from each structure. Figure 1 shows the location of the 16 samples analysed; the area covered was a few square kilometres.

The CYAMEX zone is located on the East Pacific Rise at a latitude close to 21° N. The mean spreading rate of this ridge is  $\sim$ 6 cm yr<sup>-1</sup>. In contrast with the FAMOUS area, the CYAMEX zone presents no clearly defined central valley. Detailed tectonic8, petrographic9 and geochemical10 studies are available. The area is characterized by more abundant fluid lavas than in FAMOUS, some in the form of the peculiar 'lava lakes'8. Hydrothermal circulation has been discovered in this area where the massive sulphide or manganese deposits originate11

Measurements and normalization of lead and strontium isotope ratios were completed using techniques described in refs 12, 13: the results are presented in Table 1.

In the FAMOUS area the strontium isotope values for whole rocks vary from 0.70287 to 0.70347. Such variations may a priori be ascribed to several causes. After leaching, using O'Nions and Pankhurst's<sup>14</sup> differential solution technique, samples yield values which are very close to 0.70287, in agreement with analyses by White 15,16 and O'Nions and Pankhurst14 for neighbouring areas. Therefore, the variation mentioned above can be attributed to seawater alteration and within the experimental error, the isotopic composition of Sr seems to be very constant.

The lead isotope values are rather uniform and close to ean values of  $^{206}\text{Pb}/^{204}\text{Pb} = 18.7$ ,  $^{207}\text{Pb}/^{204}\text{Pb} = 15.53$ ,  $^{208}\text{Pb}/^{204}\text{Pb} = 38.20$  with a maximum variation of 0.2 for <sup>206</sup>Pb/<sup>204</sup>Pb. Such a variation is quite small, compared with those observed for the North Atlantic as a whole<sup>17</sup> (1.9 for <sup>206</sup>Pb/<sup>204</sup>Pb). No marked difference can be observed between different types of lavas from volcanoes and fissural lava flows.

In the CYAMEX area the strontium isotope values on whole rocks and glasses are uniform and average about 0.7025 which is

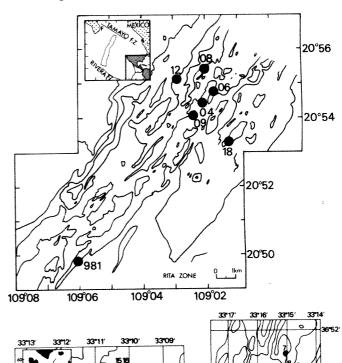


Fig. 1 Location of the FAMOUS and CYAMEX samples.

Table 1 Sr and Pb isotopic compositions of the samples studied in the FAMOUS and CYAMEX areas

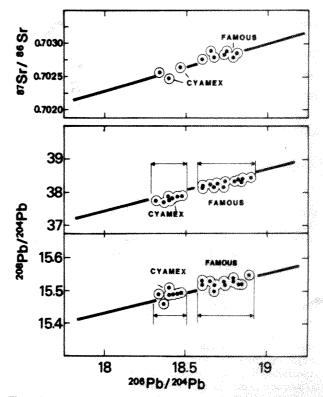
FAMOUS		<sup>87</sup> Sr/ <sup>86</sup> Sr				
Samples	$^{87}$ Sr/ $^{86}$ Sr WR	WR leached	$^{206}$ Pb/ $^{204}$ Pb	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	
ARP 74 7-5 C (IG)	$0.70292 \pm 7$	$0.70280 \pm 10$	$18.79 \pm 0.02$	$15.53 \pm 0.02$	$38.35 \pm 0.05$	1
ARP 74 7-5 S (IG)	$0.70295 \pm 7$					
ARP 74 9-12 (IG)			$18.69 \pm 0.02$	$15.52 \pm 0.02$	$38.28 \pm 0.1$	2 3
ARP 74 9-13 (IG)	$0.70295 \pm 10$	$0.70288 \pm 6$	$18.74 \pm 0.02$	$15.52 \pm 0.02$	$38.33 \pm 0.05$	
ARP 74 10-14 (IG)		$0.70289 \pm 8$	$18.65 \pm 0.02$	$15.53 \pm 0.02$	$38.28 \pm 0.05$	4 5 6
ARP 74 10-15 (IG)			$18.83 \pm 0.02$	$15.52 \pm 0.02$	$38.36 \pm 0.06$	5
ARP 74 11-11 (IG)			$18.68 \pm 0.02$	$15.52 \pm 0.02$	$38.20 \pm 0.05$	6
ARP 74 11-18 (IG)	$0.70290 \pm 6$		$18.60 \pm 0.02$	$15.53 \pm 0.02$	$38.23 \pm 0.06$	7
CYP 30-32 (IG)	$0.70302 \pm 7$	$0.70285 \pm 6$				8
CYP 31-36 (IG)	$0.70302 \pm 10$	$0.70285 \pm 6$				9
		$0.70286 \pm 6$				
CYP 31-37 (IG)	$0.70343 \pm 17$	$0.70296 \pm 6$				10
CYP 31-39 (IG)	$0.70315 \pm 7$	$0.70281 \pm 7$	$18.67 \pm 0.02$	$15.50 \pm 0.02$	$38.20 \pm 0.07$	11
ARP 73 1003 (IG)	$0.70287 \pm 10$	$0.70285 \pm 7$	$18.73 \pm 0.02$	$15.53 \pm 0.02$	$38.20 \pm 0.07$	12
ARP 74 13-21 (FZ)	an)	$0.70276 \pm 11$	$18.60 \pm 0.02$	$15.52 \pm 0.02$	$38.20 \pm 0.05$	13
ARP 74 13-24 (FZ)	viii gi		$18.89 \pm 0.02$	$15.55 \pm 0.02$	$38.45 \pm 0.06$	14
ARP 74 14-32 (FZ)	$0.70309 \pm 12$	$0.70285 \pm 8$	$18.84 \pm 0.02$	$15.52 \pm 0.02$	$38.39 \pm 0.08$	15
	$0.70312 \pm 13$		$18.79 \pm 0.02$	$15.52 \pm 0.02$	$38.31 \pm 0.05$	िस्तर विश्व हो ।
ARP 74 14-33 (FZ)	$0.70313 \pm 9$					16
CH31 DR2 301	$0.70315 \pm 7$					
CH31 DR6 325	$0.70329 \pm 17$		$18.54 \pm 0.02$	$15.55 \pm 0.02$	$38.24 \pm 0.05$	
CH31 DR 10 100	$0.70320 \pm 8$		$18.62\pm0.02$	$15.54 \pm 0.02$	$38.30 \pm 0.07$	
CYAMEX						
CYP 78 04 07		$0.70254 \pm 7$	$18.33 \pm 0.02$	$15.49 \pm 0.02$	$37.77 \pm 0.06$	
CYP 78 06 11			$18.42 \pm 0.02$	$15.49 \pm 0.02$	$37.85 \pm 0.06$	
CYP 78 09 12			$18.39 \pm 0.02$	$15.51 \pm 0.02$	$37.83 \pm 0.07$	
CYP 78 12 33			$18.37 \pm 0.02$	$15.46 \pm 0.02$	$37.73 \pm 0.05$	
CYP 78 12 35		$0.70250 \pm 9$				
CYP 78 12 36	$0.70307 \pm 6$	$0.70247 \pm 7$				
CYP 78 18 65		$0.70264 \pm 8$	$18.47 \pm 0.02$	$15.49 \pm 0.02$	$37.88 \pm 0.06$	
981 R10			$18.45 \pm 0.02$	$15.49 \pm 0.02$	$37.86 \pm 0.06$	
981 R23		$0.70247 \pm 6$	$18.39 \pm 0.02$	$15.49 \pm 0.02$	$37.83 \pm 0.06$	

We have divided the samples from the FAMOUS zone into two groups: IG, international ground; FZ fracture zone. The total blank for  $\sim$ 1 g of sample has been maintained below 1 ng for Pb and below 200 pg for Sr. WR, whole rock.

typical of the depleted mid-oceanic ridge basalts (MORB). The lead isotope values are also rather uniform but yield a less radiogenic character than for FAMOUS:  $^{206}\text{Pb}/^{204}\text{Pb} = 18.35$ ,  $^{207}\text{Pb}/^{204}\text{Pb} = 15.48$ ,  $^{208}\text{Pb}/^{204}\text{Pb} = 37.85$ .

The (Sr, Pb) or (Pb, Pb, Pb) isotope correlation diagrams (Fig. 2) show that both CYAMEX and FAMOUS data plot on the main trend defined for MORB<sup>17-19</sup>. Therefore, both zones have quite 'normal' chemical characteristics of an oceanic ridge. The uniformity of their isotopic compositions which contrasts with the variations observed in the North Atlantic<sup>16,17</sup> and with the local heterogeneities observed in peridotite xenoliths and 'high temperature' peridotites<sup>20,21</sup> has important implications. In this case, the source of basalts at the scale of 10 km is statistically homogeneous, the formation of a basaltic liquid compensates for any local minor heterogeneity in the mantle source.

Although the Pb values are roughly homogeneous, some small variations in the Pb isotope ratios remain and cannot be ascribed to secondary processes such as seawater alteration. These variations are about one-tenth of the global variation for the North Atlantic (when using the Sr-Pb correlation diagram, we may estimate the corresponding variation for the values obtained close to 0.0001, which for 87Sr/86Sr is virtually the same as those expected from the analytical error). Thus, the small variations in Pb isotopic ratios are not inconsistent with the uniformity of the Sr isotope ratios. Improvements in measuring the Sr isotopic ratio are necessary before we can study Pb-Sr correlation on such a small scale. However, on this scale these Pb isotope variations are not correlated with variations in ratios of incompatible elements like Hf/Th, Ta/Th, La/Th or La/Sm. Therefore, such small heterogeneity is not an argument against the trace element discussion made by Langmuir et al.<sup>22</sup> who



**Fig. 2** Results for CYAMEX and FAMOUS areas: a,  $(^{87}\text{Sr}/^{86}\text{Sr})$  vs  $(^{206}\text{Pb}/^{204}\text{Pb})$ ; b,  $(^{208}\text{Pb}/^{204}\text{Pb})$  vs  $(^{206}\text{Pb}/^{204}\text{Pb})$ ); c,  $(^{207}\text{Pb}/^{204}\text{Pb})$  vs  $(^{206}\text{Pb}/^{204}\text{Pb})$ .

reject the equilibrium partial melting model and propose a new model for partial melting called dynamic-melting.

We now compare the homogeneity observed on a small scale

(10 km) with that observed on a larger scale (100-1,000 km). Results from the North Atlantic<sup>3,5,17</sup>, and those on the oceanic crust of the Indian Ocean<sup>23</sup> indicate that the cause of isotopic variations obtained along the Atlantic Ridge is linked to mixing between the lower and upper mantle by injection of blobs (or hot spots). Our results show that, on a small scale, the mixing does not generate large isotopic heterogeneities but this conclusion should be evaluated in the light of the type of models discussed in ref. 18. Note that the Pb heterogeneity is larger in the case of FAMOUS than for CYAMEX. Considering that FAMOUS is on a ridge with some blobs mixing while CYAMEX is on a quite 'normal' ridge, such a difference is probably significant. With improvement of the accuracy, it will be quite interesting to study the small variations of Sr isotope ratios in these areas to decipher more precisely the processes of blob mixing and basalt genesis.

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### Palaeooceanographic significance of bottom-current fluctuations in the Southern Ocean

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Hiatuses in the sedimentary record of the Southern Ocean are usually attributed to erosion by high-velocity bottom 3. To test for palaeoclimatic mechanisms which currents 1-3 intensified bottom circulation, however, the timing of increases in bottom-current velocity must be known. As the sedimentary record of the period of initial erosion is lost within the hiatus, the timing of increased velocity must be determined in cores which are adjacent to the axis of highest velocity4.5. In those cores the accumulation rates are reduced due to the current but a record of the period of initiation of scour is preserved as a zone of particle-size winnowing of the fine-fraction3-4. The timing of major episodes of bottom-current intensification in the South Australian Basin for the past 4.5 Myr are reported here for the first time and may be used to examine the role of palaeoclimatic fluctuations on bottom-current intensity.

A series of piston cores across the axis of a high-velocity current (near a modern contour current<sup>6</sup>) which has produced hiatuses in the sedimentary record<sup>2</sup> in the South Australian Basin (Fig. 1) are used to demonstrate a method of dating erosional pulses of bottom water. These disconformities are of varying length depending on width, intensity and duration of the high-velocity bottom current responsible for scouring the sea floor. Two of the cores (E48-5 and E45-86) near the axis of flow during an expansion of the contour current have a hiatus which extends from the Miocene to latest Pleistocene while hiatuses in two adjacent cores (E49-53 and E50-2) are shorter (Fig. 2). One core (E45-21) in the area has not recorded a hiatus due to its position at the margin of the bottom water even at its greatest extent during the past 3 Myr. Because three of the cores were the site of deposition during the time span lost due to erosion in the axial-cores (Fig. 2), the particle-size distribution in those cores may be examined for evidence of winnowing which is associated with the erosion.

The mean particle-size of the non-carbonate silt fraction  $(4-62 \mu m, 8-4 \phi)$  was analysed using an electronic particle counter7 (Elzone, Particle Data). The carbonate fraction was not analysed as variations in carbonate grain-size may be affected by dissolution of carbonate rather than bottom-current velocity8. The silt fraction was analysed because the fine fraction is more sensitive to the magnitude of bottom-current velocity observed in the deep sea<sup>9,10</sup>. As the cores studied are too far from Antarctica to have a significant ice-rafted debris component the grain size of the non-carbonate silt-sized component chiefly reflects bottom-current transport. The mean silt particle size in three cores was plotted against age (Fig. 3) determined from magnetostratigraphy<sup>1,3</sup>. The mean value of the data in each core is used as an arbitrary reference level to separate periods of relatively high and low inferred bottom-water velocity (Fig. 3).

The period of erosion (or non-deposition) in core E50-2 is characterized by coarse particle sizes in the two cores (E49-53 and E45-21) marginal to the high-velocity zone (Fig. 2). Therefore, the erosional event(s) in E50-2 is 'felt' in the more distant cores by winnowing of the fine fraction. When the period of erosion or non-deposition ceased in core E50-2 the mean particle size deposited was still coarse under the waning current. In the adjacent cores, the mean particle size decreased at the same time that sedimentation resumed above the disconformity. Therefore, the marginal cores have recorded the cessation of high-velocity bottom current at ~2.6 Myr.

The initiation of the high-velocity episode may be dated at the beginning of the winnowing of particles in the marginal cores. While sediment is missing for the period 5.5-4.6 Myr, it is possible to examine the record of bottom-current velocity for the past 4.5 Myr and for a short period at ~5.5-5.6 Myr (Fig. 3). The first episode of high-velocity is marked by a coarsening of the mean particle size at ~4.3 Myr which persisted until ~3.8 Myr. Several shorter pulses with lower magnitude followed the initial coarsening of mean particle size and may record periods of current intensification which either eroded or inhibited deposition at the site of core E50-2. The episodic nature of the inferred bottom-water velocity fluctuations imply that sediment was deposited and subsequently eroded in the scour zone so that the hiatus is a result of multiple high-velocity events.

Unfortunately, the bottom-current velocity record of the lower Gilbert Chron cannot be examined due to the lack of recovery of sediment of that age in the South Australian Basin<sup>2</sup>. If, however, the initial increase in bottom-current velocity occurred at 3.8-4.3 Myr, then ~1 Myr of sediments were eroded at the site of core E50-2 (Fig. 3). Based on average sedimentation rates after the disconformity of 0.35 cm kyrcores E50-2 and E45-21, the episodes of increased velocity eroded ~3.5 m of sediment deposited before 4.3 Myr and 5 m was eroded and/or not deposited from 4.3 to 2.6 Myr. The total erosional effect of the bottom currents in this area, therefore, is nearly 10 m of sediment. Areas of more intense erosion have occurred in the southeastern Indian Ocean as much older sediment is exposed by other disconformities1.2 which must have been nearer the axis of flow.

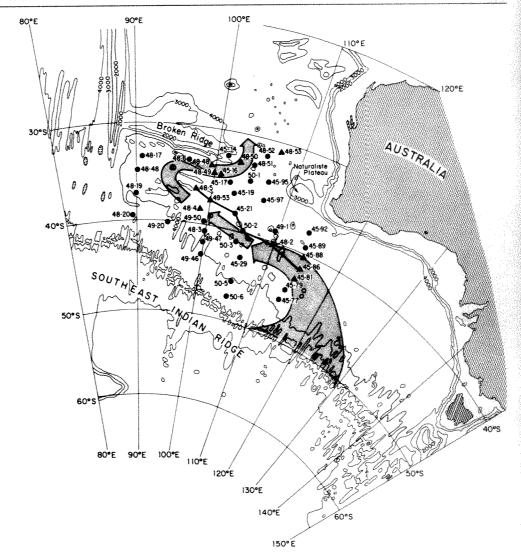


Fig. 1 Map of South Australian Basin showing major physiographic features, bathymetry and Eltanin core locations<sup>3</sup>. Arrows indicate an inferred bottom-water path based on the presence of cores with hiatuses (△)<sup>2</sup>. ♠, Cores without hiatuses<sup>2</sup>. The pathway is near a modern contour-current identified from benthic foraminiferal assemblages<sup>6</sup>. Profile refers to cores in Fig. 2.

A second unconformity in the area is recorded in core E49-53 (ref. 2). The base of the hiatus is at  $\sim 1.35$  Myr (Fig. 2) and the overlying sediment is known to be < 0.72 Myr because it is all within the normal polarity Brunhes Chron (Fig. 2). Much of the period represented by the hiatus in E49-53 is characterized by high inferred bottom-current velocity in cores E45-21 and E50-2 (Fig. 3). An episode of high velocity is recorded from 1.4 to 0.75 Myr. As the high velocity event began near the time of the base of the hiatus, there may have been no erosion but just winnowing and non-deposition of sediment. Sediment above the disconformity may fall anywhere within the period of low-velocity which encompasses the Brunhes Chron and no attempt was made to determine palaeovelocity fluctuations as no age could be assigned.

The history of AABW velocity may be examined in detail for the first time. The period of increased velocity 3.8-4.3 Myr in the South Australian Basin corresponds to an increased hiatus frequency throughout the south-east Indian Ocean (Fig. 4). The increase in bottom-water velocity in the late Gilbert Chron occurred at the same time as a major cooling in the Antarctic region 12-14. Fluctuations observed in the temperature of the sub-Antarctic and Antarctic region 12 closely match the pulses in bottom-water velocity with cold climatic conditions corresponding to increased velocity. When climatic conditions in the sub-Antarctic and Antarctic warmed in the Gauss Chron 13, the bottom-current velocity waned. The increased benthic circulation with Antarctic region refrigeration occurred due to either increased thermohaline circulation or increased wind stress on the Antarctic circumpolar current; either mechanism could result from steepened Equator to poleward temperature gradients. The Northern Hemisphere refrigeration which resul-

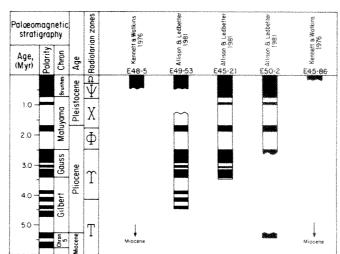


Fig. 2 Magnetostratigraphy of five cores on a profile across the path of a bottom-current in the Australian Basin (see Fig. 1). The disconformities in these cores may be traced into cores E49-53, E45-21 and E50-2 where winnowing of the grain-size population may be used to date the initiation of high velocity events which created a scour zone.

ted in the ice ages, however, occurred at 3.2 Myr (ref. 15) at a time when Antarctic bottom-current circulation waned in the southeastern Indian Ocean (Figs 3, 4), the South Atlantic Ocean<sup>4</sup> and South Pacific Ocean<sup>16</sup>.

The decreased Antarctic-source bottom circulation at the time of the initiation of the Quaternary glaciations may have

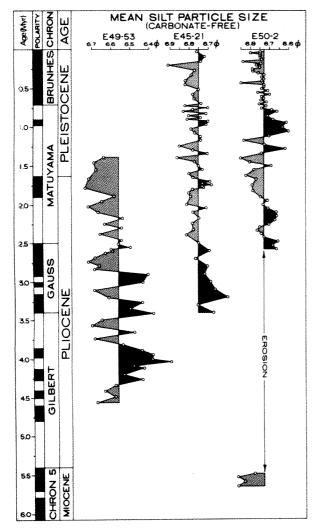


Fig. 3 The mean particle size of the carbonate-free silt fraction in cores E49-53, E45-21 and E50-2 is plotted against age. Blackened areas represent highest relative inferred bottom-water velocity. The disconformity in E50-2 is synchronous with a winnowing zone in E49-53 and E45-21 which is characterized by coarser particle sizes. Highest inferred bottom-water velocity occurred in the late Gilbert and Gauss Chrons. The initiation of winnowing in core E49-53 at 4.3 Myr marks the beginning of the erosion in core

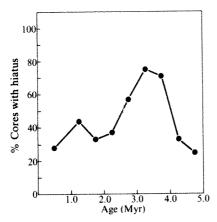


Fig. 4 The percentage of all cores >4440 m in the south-east Indian Ocean 1.2.18 is  $\ge 2.5-4$  Myr BP. The increase in hiatus frequency at ~4 Myr is nearly synchronous with an inferred increase in bottom-current velocity in core E49-53 and the decrease at ~2.5 Myr is nearly synchronous with a decrease in bottom-current velocity inferred in three cores (see Fig. 3).

important palaeooceanographic implications. In the modern ocean, North Atlantic deep water (NADW) provides the salinity component of AABW which increases the density of the cold current to the point where it is the densest water-mass in the ocean. If the production of NADW is affected by the ice ages, then a direct effect may occur in AABW production rates. Two effects may be postulated, however. Cooler Northern Hemisphere climates might increase NADW production through increased sea-ice production in the Norwegian Sea. The cooler climates, however, may result in long periods of thickened sea-ice which halt NADW production by limiting the reservoir for cold saline water within the Norwegian Sea. The latter method has been postulated for Pleistocene glaciations<sup>17</sup> and may be responsible for the decrease of AABW at the onset of the ice ages.

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### C<sub>27</sub>-C<sub>29</sub> ring A monoaromatic steroids in Cretaceous black shales

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The structural elucidation of aromatic steroid hydrocarbons, which are widespread constituents of sediments and petroleums, has been attempted<sup>1</sup>; several series of triaromatic steroids have been identified<sup>2,3</sup> and a structural hypothesis for the major series of the ubiquitous ring C monoaromatic steroids has been proposed<sup>2,4</sup>. Although many of these monoaromatic compounds are already present in ancient shales of a low degree of maturity, the stage at which they form and the mechanism of their aromatization are still unclear. Early aromatization of ring A of steroids has been suspected<sup>5.6</sup>, but never confirmed. Here we report the elucidation of the structures of two series of ring A monoaromatic steroids, 1 and 2, which occur in immature Cretaceous black shales from the Southern and Eastern North Atlantic, collected during the Deep Sea Drilling Project (DSDP). Compounds 1 and 2 were identified as major constituents of the aromatic fractions of these samples by comparison with authentic standards.

Steroid derivatives have been widely used as geochemical markers to study chemical transformations at various stages of diagenesis or maturation in the subsurface. There is good evidence for early alterations of the steroid skeleton in recent or immature ancient sediments: microbial reduction of the  $\Delta^5$ double bond, dehydration of stanols leading to  $\Delta^2$ -sterenes and the subsequent formation of steranes or isomerization of

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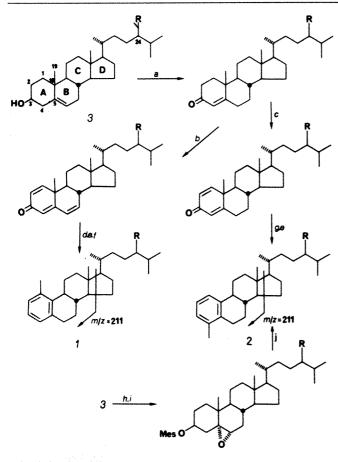


Fig. 1 Scheme of synthesis of ring A monoaromatic steroids, after refs 15-17. a, Cyclohexanone, Al(O-iPr)<sub>3</sub>; b, chloranil, pentanol; c, DDQ, dioxane; d, iPr-OH, Al(O-iPr)<sub>3</sub>; e, H<sup>+</sup>; f, H<sub>2</sub>, Pd/C; g, LiAlH<sub>4</sub>; h, CH<sub>3</sub>SO<sub>2</sub>Cl; i, m-ClC<sub>6</sub>H<sub>4</sub>CO<sub>3</sub>H; j, CH<sub>3</sub>CO<sub>2</sub>H, HBr 48%.

sterenes<sup>7-11</sup>. However, the aromatization reactions of this class of molecules, which is widespread in living organisms, in particular in phytoplankton, have been little studied<sup>1</sup>, despite the fact that the distributions of aromatic steroids are being increasingly used, as a fingerprint, in problems of maturation of geological organic matter<sup>3</sup>, as well as for correlating crude oils and their source rocks<sup>12</sup>.

The black shale samples (10-20 g) were obtained from DSDP Legs 40 (sites 364 and 365) and 41 (site 367) in the Angola and Cape Verde basins. These shales have been deposited in anoxic environments; their organic carbon content is high, ranging from 16 to 5% (refs 13, 14). The samples were extracted with chloroform and total extracts were separated by column and TLC over silica gel. The content of total aromatic hydrocarbons ranged between 17 and 5% of the extract. The mono+ diaromatic hydrocarbons were further purified by HPLC (SiO<sub>2</sub>, 10 µm; heptane) as described previously<sup>2</sup> and analysed by computerized gas chromatography-mass spectrometry (GC-MS; LKB 9000 S). Steroid hydrocarbons bearing on aromatic A or B ring display a major fragmentation at m/z = 211, which can be used for the study of their distribution by mass fragmentography of this specific ion (Fig. 1). The mass fragmentograms of the molecular ions at m/z = 366, 380 and 394 show a very similar distribution in which at least four major series are apparent (Fig. 2).

Compounds 1 (R = H) and 2 (R = H; R =  $C_2H_5$ ) were prepared from cholest-5-en-3 $\beta$ -ol (cholesterol; 3, R = H) and 24(R) -ethylcholest-5-en-3 $\beta$ -ol (sitosterol; 3, R =  $C_2H_5$ ) as a starting material (Fig. 1)<sup>15-17</sup>. Their structural data agree with those of the literature<sup>15,16,18</sup> and were confirmed by proton magnetic resonance and mass spectrometry.

1 R = H 1-methyl-19-nor-cholesta-1,3,5(10)-triene NMR (250 MHz; CDCl<sub>3</sub>;  $\delta$  p.p.m. JHz): 0.75 (3H,s); 0.87 (6H,d,J = 6.4); 0.93 (3H,d,J = 6.4); 2.29 (3H,s);6.69-6.85 (3H,m). MS (70 eV): m/z = 366 (M<sup>+</sup>, 95%); 351 (18%); 253 (M<sup>+</sup> - C<sub>8</sub>H<sub>17</sub>; 4%); 226 (17%); 211 (100%); 197 (12%); 183 (12%); 170 (34%); 158 (42%); 157 (64%); 143 (35%); 131 (55%).

2 R = H 4-Methyl-19-nor-cholesta-1,3,5(10)-triene NMR (250 MHz; CDCl<sub>3</sub>;  $\delta$  p.p.m., JHz): 0.71 (3H,s); 0.88 (6H,d,J = 6.4) 0.95 (3H,d,J = 6.4); 2.17 (3H,s); 6.83-7.04 (3H,m). MS (70 eV): m/z = 366 (M<sup>+</sup>, 85%); 351 (2.5%); 253 (M<sup>+</sup> - C<sub>8</sub>H<sub>17</sub>; 2%); 226 (15%); 211 (100%); 197 (8%); 183 (6%); 170 (10%); 158 (65%); 157 (32%); 143 (20%); 131 (36%).

R = C<sub>2</sub>H<sub>5</sub> 4-Methyl-24-ethyl-19-nor-cholesta-1,3,5(10)-triene NMR (250 MHz; CDCl<sub>3</sub>; δ p.p.m., JHz); 0.71 (3H,s); 0.83 (3H,d,J = 6.8), 0.85(3H,d,J = 6.8), 0.86(3H,t,J = 6.8); 0.95(3H,d,J = 6.4); 2.16 (3H,s); 6.83-7.04 (3H,m). MS (70 eV): m/z = 394 (M<sup>+</sup>, 85%); 379 (2.5%); 253 (M<sup>+</sup>-C<sub>10</sub>H<sub>21</sub>; 5%); 226 (13%); 211 (100%); 197 (8%); 183 (6%); 170 (10%); 158 (66%); 157 (32%); 143 (16%); 131 (34%).

These compounds were identified with those occurring in the geological samples by comparison of the mass spectra and coelution in single ion mass fragmentography GC-MS on two glass capillary columns (SE 30; SP 2250, Supelco, 25 m× 0.25 mm). Compounds 1 (R = CH<sub>3</sub>; C<sub>2</sub>H<sub>5</sub>) and 2 (R = CH<sub>3</sub>) were tentatively identified on the basis of their mass spectra and retention times.

The immature character of these Cretaceous black shales  $^{13.14}$  is confirmed by the presence of the less stable  $\beta\beta$ -hopane series as major constituents of the alkane and acid fractions, as well as an important fraction of unsaturated hydrocarbons in which monosterenes and hopenes are largely predominant (B.C. and P.A., unpublished data). The identification of monoaromatic steroids 1 and 2 shows that the aromatization of ring A of the precursor sterols begins at a very early stage of diagenesis. These results also demonstrate a novel pathway of aromatization of sterols in the subsurface, starting in ring A rather than in ring C

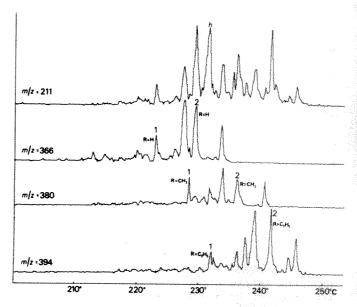


Fig. 2 Mass fragmentograms of ions 211, 366, 380 and 394 showing the distribution of ring A monoaromatic steroid hydrocarbons in a Cretaceous black shale from the DSDP (Leg 40, site 364). Numbers refer to structures in Fig. 1. h, Monoaromatic C<sub>27</sub> hopanoid. Conditions: SP 2250, 25 m × 0.25 mm, 0.08 μm, 140–250 °C, 2 °C min<sup>-1</sup>. Computerized GC-MS, LKB 9000 S.

as previously described<sup>1,2,4</sup>. Note that only traces of ring C monoaromatic steroids have been detected in our samples which mostly correspond to confined reducing environments. Ring C aromatization obviously needs further isomerization of the precursor sterenes, which seem to be favoured by a more open and oxidizing environment (B.C. and P.A. unpublished data).

The geochemical aromatization reaction proceeds with a shift of the C-19 methyl group of the precursor sterols from position 10 to position 1 or a preferred rearrangement to position 4, both reaction pathways being similar to the action of acids on various unsaturated steroid derivatives in the laboratory 15,19. Loss of the angular methyl group seems to be negligible in our case, as indicated by a weak m/z = 197 mass fragmentogram. The aromatization process of ring A of sterols could well be due, in its final steps, to an acid catalysis of minerals, clays in particular, acting in the sediments at an early stage of diagenesis. The cholestatrienes leading to the aromatic hydrocarbons 1 and 2 could have been formed by microbial and chemical transformations of the parent sterols<sup>8,20</sup>. As ring A monoaromatic steroids have not yet been observed in significant amounts in more mature sediments, they are probably intermediates of degradation of steroids restricted to early diagenesis, leading later to triaromatic steroids.

Work is in progress on the identification of the other series of monoaromatic or triunsaturated steroid hydrocarbons occurring in geological samples.

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### $N\alpha$ -acetylation is linked to $\alpha$ -MSH release from pars intermedia of the amphibian pituitary gland

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Most amphibians can adapt their colour to that of their background by a mechanism which is controlled by melanophore stimulating hormone (MSH), a product of the pars intermedia of the pituitary gland1. Although the melanotropic peptides of amphibians have never been sequenced, immunological evidence indicates that at least some of them are structurally related to  $\alpha$ -MSH<sup>2-4</sup>. In our studies of the biosynthesis and structure of melanotropic peptides in the pars intermedia of Xenopus laevis, we have found evidence, reported here, for des-N $\alpha$ -acetyl- $\alpha$ -MSH as the immediate precursor of  $\alpha$ -MSH, with the acetylation taking place just before or during release from the pituitary.

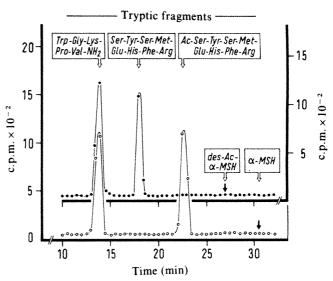


Fig. 1 Tryptic peptide mapping by HPLC of newly synthesized des- $N\alpha$ acetyl- $\alpha$ -MSH ( $\odot$ ) and  $\alpha$ -MSH ( $\odot$ ). Neurointermediate lobes of black-adapted X. laevis were incubated for 4.5 h at 22 °C in 50  $\mu$ l incubation medium (for composition see ref. 5) containing 20 µCi each of <sup>3</sup>H-lysine, <sup>3</sup>H-tyrosine, <sup>3</sup>H-phenylalanine and <sup>3</sup>H-tryptophan (specific activities 90, 50, 80 and 26 Ci mmol<sup>-1</sup>, respectively; Amersham). Lobes were extracted with 500 µl 0.1 M HCl, the extract was analysed with HPLC, and the newly synthesized product co-eluting with synthetic des- $N\alpha$ -acetyl- $\alpha$ -MSH was isolated from the lobes. To the incubation medium 500  $\mu$ l 0.1 M HCl were added, the acidified medium was analysed with HPLC and the newly synthesized product co-eluting with synthetic α-MSH was isolated from the incubation medium. Tryptic digestion of the isolated peptides (to which 20  $\mu$ g bovine serum albumin were added) was performed at 37 °C for 2 h with 10  $\mu$ g trypsin (DPCC-treated; Sigma) in 10  $\mu$ l 55 mM HEPES buffer (pH 8.0); the digest was analysed with HPLC. Digestion of the radioactive tryptic fragments to free amino acids revealed that the 14-min fragments contained radioactive tryptophan and lysine, and both the 18- and 22.5-min fragments contained radioactive tyrosine and phenylalanine. Black arrows indicate the elution positions of the undigested radioactive peptides. For comparison, 50 µg synthetic des- $N\alpha$ -acetyl- $\alpha$ -MSH (a gift from Dr G. I. Tesser, Department of Organic Chemistry, Nijmegen) and 50 µg α-MSH (Peninsula) were digested with 10 µg trypsin in the same conditions as described for digestion of the radioactive peptides, except no bovine serum albumin was added. The tryptic fragments of the synthetic peptides (shown in upper boxes) were detected with a post-column derivatization system, using o-phthaldialdehyde (Sigma) as a fluorogenic reagent, and they were further identified with specific staining techniques for the amino acids histidine and tryptophan according to ref. 20. The elution positions of the undigested synthetic peptides are also indicated (lower boxes). HPLC separation was on Spherisorb 10 ODS (Chrompack, 250×4.6 mm i.d.) using a stepwise gradient with a flow rate of 2 ml min<sup>-1</sup>, and 0.5-min fractions were collected. The gradient was established with 0.5 M formic acid, 0.14 M pyridine (pH 3.0) as the primary solvent and 1-propanol as the secondary solvent

We have previously shown by in vitro pulse-chase techniques coupled to HPLC analysis that the biosynthesis of peptides in the pars intermedia of X. laevis begins by the production of a large protein which is subsequently processed to a number of peptides5, three of which possess melanotropic activity. One of them has since been shown to co-migrate with synthetic des- $N\alpha$ acetyl- $\alpha$ -MSH on acid-urea gels and to have identical retention times to the synthetic peptide during HPLC analysis with several different elution gradients (unpublished observations). Tryptic digestion of this newly synthesized peptide yielded two fragments (Fig. 1) with elution times identical to those of the two fragments obtained from the tryptic digest of synthetic des- $N\alpha$ acetyl- $\alpha$ -MSH. Moreover, the radioactive amino acid content of the tryptic fragments was in agreement with that expected on the basis of the amino acid content of the comparable fragments of the synthetic peptide. Therefore, this newly synthesized melanotropic product is des- $N\alpha$ -acetyl- $\alpha$ -MSH. Another melanotropic peptide co-eluted from the HPLC column with synthetic  $\alpha$ -MSH and co-migrated with this peptide during acid-urea gel electrophoresis. The elution times of the two radioactive tryptic fragments of this newly synthesized product (Fig. 1) corresponded exactly with those of the two tryptic fragments of synthetic  $\alpha$ -MSH. Furthermore, the radioactive amino acid content of each tryptic fragment of this product was in full agreement with its identity as  $\alpha$ -MSH. The designation of the two newly synthesized products is supported by the observation that the only structural difference between them is located on the N-terminus.

Pulse-chase analysis of the biosynthesis of des- $N\alpha$ -acetylα-MSH and α-MSH revealed a likely precursor-product relationship for these peptides. During the first 2 h des- $N\alpha$ acetyl- $\alpha$ -MSH accumulated rapidly, then gradually decreased. α-MSH was barely demonstrable in the first 2 h and gradually increased thereafter, concomitant with the decrease of des-Naacetyl- $\alpha$ -MSH (Fig. 2). A further observation was that des- $N\alpha$ acetyl-\alpha-MSH was almost completely restricted to the tissue while  $\alpha$ -MSH was exclusively found in the medium. Additional experiments were performed to establish that the absence of des-Nα-acetyl-α-MSH in the medium was not the consequence of extracellular acetylation or rapid degradation of this peptide. Isolated radioactive des-Na-acetyl-a-MSH, incubated with neurointermediate lobes, remained in the non-acetylated form and was stable for at least 8 h. Moreover, superfusion of neurointermediate lobes, whereby any possible modification or proteolysis of secreted peptides was prevented by collecting the perfusate in solutions containing acid or enzyme inhibitors, demonstrated that over 95% of the newly synthesized MSH released was in the acetylated form.

In amphibians, the physiological factor inhibiting MSH release is thought to be dopamine<sup>6,7</sup>. In Xenopus, this cate-cholamine inhibits the in vitro release of all newly synthesized peptides<sup>8–10</sup>. To investigate the effects of inhibition of release on the acetylation process we conducted pulse-chase experiments with dopamine present during the chase. In the control experiment, ~40% of the newly synthesized MSH was in the  $\alpha$ -MSH form and, as expected, this acetylated product was found exclusively in the medium (Fig. 3a). The des-N $\alpha$ -acetyl- $\alpha$ -MSH was found almost exclusively in the tissue. In the dopamine-treated group, release of newly synthesized peptides was completely inhibited and all MSH in the tissue was des-N $\alpha$ -acetyl- $\alpha$ -MSH (Fig. 3b). Thus, inhibition of release was accompanied by inhibition of acetylation.

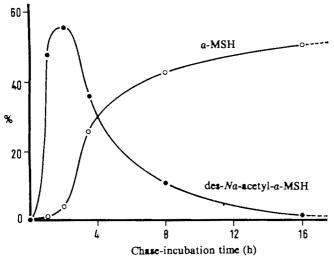


Fig. 2 Levels of newly synthesized des- $N\alpha$ -acetyl- $\alpha$ -MSH and  $\alpha$ -MSH as a function of chase-incubation time. *Xenopus* neurointermediate lobes were presentated for 1 h before a 30-min pulse incubation in <sup>3</sup>H-tryptophan, followed by chase incubations in normal incubation medium for 1, 2, 3 5, 8 or 16 h After the pulse-chase incubations, lobe extracts and incubation media were analysed with HPLC. With the gradient used, newly synthesized des- $N\alpha$ -acetyl- $\alpha$ -MSH and  $\alpha$ -MSH eluted at 22.5 mm and 28 mm, respectively. To quantify the relative amounts of these peptides, the chromatographic profile between 20 and 30 min elution time was used. For each chase group, the total level of radioactivity (lobe plus medium) was determined by adding the c p m. values of the HPLC fractions. The radioactivity of each HPLC fraction was then expressed as a percentage of this total level. In the figure, the percentages represent the sum of the percentage found in the lobe and that in the medium for des- $N\alpha$ -acetyl- $\alpha$ -MSH ( $\Theta$ ) or  $\alpha$ -MSH (O) at each chase-incubation time.

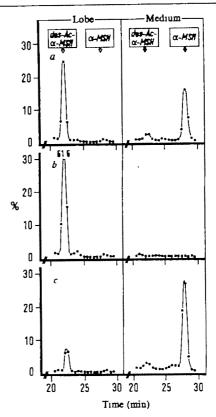


Fig. 3 Effect of dopamine and cyclic AMP on the release of newly synthesized des-N $\alpha$ -acetyl- $\alpha$ -MSH and  $\alpha$ -MSH. Lobes were premcubated for 1 h before a 30-min pulse with <sup>3</sup>H-tryptophan, followed by chase incubations of 3.5 h m control incubation medium (a), medium containing  $10^{-5}$  M dopamine (3-hydroxytyramine HCl; Sigma) (b), or medium containing 6 mM cyclic AMP (8-bromo cyclic AMP, Sigma) (c). The HPLC fractions are expressed in percentages using the method described in Fig. 2 legend.

Cyclic AMP stimulates release of newly synthesized peptides from the pars intermedia of  $Xenopus^{10}$ . The present study shows that cyclic AMP treatment enhanced the amount of  $\alpha$ -MSH (Fig. 3c). Approximately 75% of the newly synthesized MSH was present in the  $\alpha$ -MSH form and, again, this form was found exclusively in the medium. Thus, stimulation of release was accompanied by stimulation of the acetylation process. Altogether, our results indicate that the acetylation of des- $N\alpha$ -acetyl- $\alpha$ -MSH occurs immediately before or during release.

The above results, dealing with newly synthesized peptides, suggest that  $\text{des-}N\alpha\text{-acetyl-}\alpha\text{-MSH}$  could constitute a 'storage-form' of the hormone. This contention is supported by observations we made concerning stored peptides (unpublished data). Using bioassays, we demonstrated that the pars intermedia of white-adapted *Xenopus*, which is known to store melanotropic peptides<sup>3</sup>, contains ~10 times more des- $N\alpha$ -acetyl- $\alpha$ -MSH than  $\alpha$ -MSH. Further, infusing animals adapting to a white background with <sup>3</sup>H-lysine resulted in a preferential accumulation of radioactive des- $N\alpha$ -acetyl- $\alpha$ -MSH such that after 10 days >90% of the radioactive MSH in the pars intermedia was in the non-acetylated form.

Des- $N\alpha$ -acetyl- $\alpha$ -MSH shows considerably less melanotropic activity than  $\alpha$ -MSH<sup>11</sup>. That acetylation of peptide hormones need not always be accompanied by an increase in activity is, however, illustrated by the fact that  $\beta$ -endorphin is not biologically active in its acetylated form<sup>12</sup>. It is interesting that  $\alpha$ -MSH and  $\beta$ -endorphin are derived from the same prohormone<sup>13,14</sup>. Whether acetylation constitutes a control point in regulating the biological potency of pars intermedia peptides, as suggested earlier<sup>12</sup>, remains to be established.

In the rat pituitary gland, Woodford and Dixon<sup>15</sup> demonstrated the presence of  $N\alpha$ -acetyltransferase, which can acetylate the  $\alpha$ -amino group of synthetic des- $N\alpha$ -acetyl- $\alpha$ -MSH to form  $\alpha$ -MSH. The acetylation reaction reported in the present investigation is probably catalysed by such an enzyme. As this

acetylation is closely related to the release process, the enzyme is probably associated with the secretory granules or with the cell membrane. This situation would be unique, as most acetylations of polypeptides take place before or during packaging in the secretory granules 12,16; a pertinent example is the  $N\alpha$ -acetylation of  $\beta$ -endorphin in the rat pars intermedia<sup>17</sup>

The process of exocytosis implies the fusion of the membrane of the secretion granule with the plasma membrane1 Membrane fusion, in general, may be accompanied by physical and chemical changes in the membranes involved<sup>19</sup>. An intriguing possibility for the acetylation of des- $N\alpha$ -acetyl- $\alpha$ -MSH in the amphibian pars intermedia is that during the secretory process physicochemical changes in the microenvironment near the cell membrane lead to activation of a specific  $N\alpha$ -acetyltransferase, thus linking acetylation of the hormone to its release.

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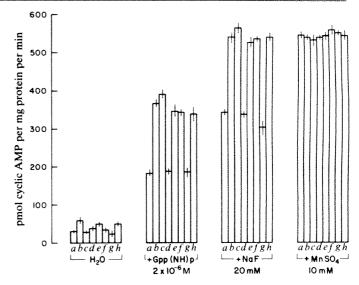
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#### The regulatory subunit of adenylate cyclase interacts with cytoskeletal components

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The adenylate cyclase system, which mediates cellular responses to a variety of hormones and neurotransmitters, contains at least three plasma membrane-associated proteins: the hormone receptor, the regulatory or guanyl nucleotide-binding unit (G unit) and the catalytic moiety. Activation of the enzyme after binding of hormone to the receptor involves binding of GTP to the G unit1. Activation can be enhanced by increasing the fluidity of the membrane with unsaturated fatty acids2 with the local anaesthetic prilocaine3, and perhaps, in vivo, by phospholipid methylation<sup>4</sup>. Disruption of microtubules increases localized mobility of membrane proteins in a manner similar to that of unsaturated fatty acids<sup>5</sup> and can enhance cyclic AMP accumulation in intact leukocytes<sup>6-9</sup>. We therefore decided to investigate whether there is a direct interaction between microtubules and adenylate cyclase. We show here that colchicine, vinblastine and cis-unsaturated fatty acids enhance G unitmediated activation of adenylate cyclase, implying that microtubules or tubulin are involved in the attachment of the G unit to the membrane.



Incubated (before assay) with:

Fig. 1 Adenylate cyclase activity in synaptic membranes preincubated with fatty acids or microtubule-disrupting agents. Enriched synaptic membrane fractions (ESMF) were prepared from 21-day old male Sprague-Dawley rats by a modification  $^{25}$  of the method of Gray and Whittaker  $^{26}$ . After washing the 10,000~gpellet three times in a 0.32 M sucrose, 20 mM Tris-maleate pH 7.8, 5 mM MgSO<sub>4</sub>, 2 mM EGTA, 1 mM dithiothreitol (DTT) buffer, the membranes (about 80-85% synaptosomes by electron microscopy) were stored under liquid nitrogen. After thawing, synaptosomes were lysed in 20 mM Tris-maleate pH 7.8, 5 mM MgSO<sub>4</sub>, 1 mM DDT (1.2 mg protein per ml) and treated at 30 °C, 20 min with compounds a-h as indicated. 40 µl of the membrane suspension were then incubated (30 °C, 20 min) with 10 µl of Gpp(NH)p, NaF, Mn<sup>2+</sup> or H<sub>2</sub>O. Adenylate cyclase assays<sup>25</sup> are started by the addition of ATP and a regenerating system (100  $\mu l$ total volume), carried out for 10 min at 30 °C and stopped by boiling. Cyclic AMP produced is assayed by protein binding<sup>27</sup> using a rabbit skeletal muscle-binding protein<sup>28</sup>. Values are the means of triplicate determinations in one of four similar experiments. a,  $H_2O$ ; b, colchicine  $10^{-6}$  M; c, vinblastine  $10^{-6}$  M; d, lumicolchicine  $10^{-5}$  M; e, cis-vaccenic acid  $10 \,\mu g \, \text{ml}^{-1}$ ; f, linoleic acid  $10 \,\mu g \, \text{ml}^{-1}$ ; g, stearic acid  $10 \,\mu g \, \text{ml}^{-1}$ ; h, linoleic acid + colchicine.

In enriched synaptic membrane fractions (ESMF) from rat cerebral cortex, the G unit (with GTP) can interact directly with the catalytic moiety to activate adenylate cyclase. In this case, the hormone receptor is functionally bypassed 10,11 and the interaction of two, rather than three, subunits can be examined while the regulatory role of the G unit is maintained. Thus when fluoride or guanylylimidodiphosphate (Gpp(NH)p-a hydrolysis-resistant GTP analogue) is present, adenylate cyclase is stably activated by the G unit-catalytic moiety complex. Manganese, however, can bypass the G unit to activate the catalytic moiety directly12.

In an attempt to increase the G unit-catalytic moiety interaction by increasing the mobility of the proteins, ESMF were incubated with colchicine ( $K_a 5 \times 10^{-7} \text{ M}$ ) or vinblastine and the effects of these agents on Gpp(NH)p or fluoride activation of adenylate cyclase was examined. In these conditions, adenylate cyclase activity is significantly increased (Fig. 1). Lumicolchicine, which does not bind to tubulin<sup>13</sup>, does not have this effect at any concentration. Incubation with membrane fluidizers such as cis-vaccenic or linoleic acid is approximately as effective as colchicine or vinblastine in elevating Gpp(NH)p or NaF activation of adenylate cyclase. The effects of fatty acids and colchicine are not additive. When fatty acids, colchicine or vinblastine were added, they did not alter the catalytic activity as determined by activity in the presence of manganese.

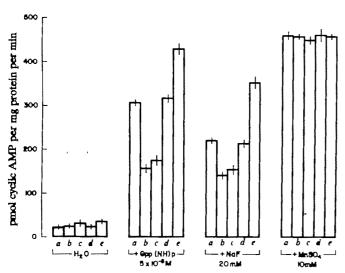
<sup>\*</sup> Author to whom correspondence should be addressed at: Department of Neurology, Yale University School of Medicine, New Haven, Connecticut 06510, USA.

Membranes that have been incubated with colchicine or vinblastine, and subsequently washed, show a loss of Gpp(NH)p or NaF-mediated adenylate cyclase activity. Treatment of ESMF with cis-vaccenic acid, however, continues to enhance adenylate cyclase activity even after the membranes have been washed. Again the intrinsic catalytic activity, as measured with manganese, was not altered by these treatments (Fig. 2).

To test whether any material that may have been stripped from the membrane during colchicine preincubation would restore or enhance Gpp(NH)p and fluoride sensitivity, we added back concentrated, dialysed supernatants from the washes following preincubation with colchicine. We found that both the control supernatant (data not shown) and, to a greater extent, the colchicine preincubation supernatant (dialysed free of colchicine and guanyl nucleotide) augmented adenylate cyclase activity (both basal and in the presence of Gpp(NH)p or fluoride: Flg. 3). These data suggest that the G unit may be liberated from the synaptic membrane.

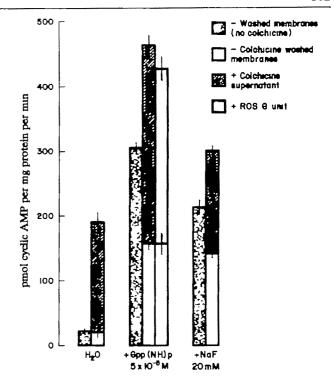
Previous experiments in this laboratory have indicated that the GTPase complex (G unit) from amphibian or bovine photoreceptor rod outer segment cyclic GMP phosphodiesterase (ROS-PDE) is similar to the G unit of adenylate cyclase <sup>14</sup>, and that ESMF adenylate cyclase can be activated by photoreceptor components<sup>15</sup>. The ROS G unit becomes a stable activator of ROS-PDE or adenylate cyclase when Gpp(NH)p is bound in place of GTP<sup>16,17</sup>. Thus, when purified preparations of ROS G unit are added to ESMF which have been depleted of guanyl nucleotide or fluoride responsiveness by colchicine, Gpp(NH)p and NaF responsiveness is restored. The degree of restoration is comparable with that observed when concentrated supernatants from membranes preincubated with colchicine and subsequently washed (colchicine/wash supernatant) are added to ESMF (Fig. 3).

While the ability to add back Gpp(NH)p and fluoride responsiveness to colchicine-treated ESMF gives a qualitative indica-



Adenylate cyclase assayed in the presence of.

Fig. 2 Preincubation of synaptic membranes with microtubule-disrupting agents or fatty acids followed by washing effects on adenylate cyclase. ESMF were treated (as in Fig. 1) with the indicated compounds and then washed twice with 10 volumes of 10 mM Tris-maleate pH 7.8, 2 mM EDTA, 1 mM DTT followed by centrifugation at 10,000g for 15 min (4 °C). Membranes were resuspended and assayed for adenylate cyclase in the presence of H<sub>2</sub>O, Gpp(NH)p, NaF or MnSO<sub>4</sub> as noted for 10 min at 30 °C. Values are means of triplicate determinations for one of three similar experiments. Fluoride activation is lower than that shown in Fig. 1, as NaF is present only during the assay step<sup>25</sup>. a, No addition; b, colchicine 5×10<sup>-6</sup> M; c, vinblastine 5×10<sup>-6</sup> M; d, humicolchine 10<sup>-5</sup> M; e, cts-vaccenic acid 10 μg ml<sup>-1</sup>.



Adenylate cyclase assayed in the presence of:

Fig. 3 Reconstitution of adenylate cyclase activity with soluble components. Supernatants were prepared by incubating ESMF from 12 cerebral cortices with  $10^{-5}\,\mathrm{M}$  Gpp(NH)p ( $\pm5\times10^{-6}\,\mathrm{M}$ colchicine) for 20 min at 30 °C and washing with 10 mM Trus-maleate pH 7.8, 2 mM EDTA, 1 mM DTT. The process was repeated twice and the three 10,000g supernatants pooled and centrifuged for 1 h at 105,000g. The resulting supernatant was concentrated and dialysed free of Gpp(NH)p or colchicine in an Amicon Cf 25 cone (molecular weight cut-off ~25,000) in 20 mM Tris-maleate pH 7.8, 5 mM MgSO<sub>4</sub>, 1 mM DTT. 10 µl of a 0.74 mg protein per mi solution were added where indicated. ROS G unit was prepared (as described elsewhere  $^{29}$ ) by exposing toad (Bufo marinus) ROS to light and  $2 \times 10^{-6}$  M Gpp(NH)p. The ROS G unit consists of three polypeptides with apparent molecular weights of 39,000, 37,000 and 10,000, and these three species comprise >99% of the material added. The supernatant was concentrated (as above for ESMF) and 10 µl of a 0.12 mg protein per ml solution were added where indicated. Both the ROS G unit and the ESMF supernatants are devoid of adenylate cyclase and PDE activity, and do not alter the PDE activity of the ESMF.

tion that G unit activity can be lost and replaced, it is necessary to quantify the replacement. Towards this end, endogenous G unit activity was depleted with differential heat inactivation<sup>18</sup>. When ESMF are heated at 42 °C for 30 min, about 15% of Gpp(NH)p or NaF-mediated adenylate cyclase activity is retained compared with about 54% of catalytic activity (as monitored with Mn<sup>2+</sup>). Colchicine/wash supernatant or ROS G unit are capable of restoring the Gpp(NH)p and NaF response of heated membranes to a level concomitant with that of remaining catalytic activity (Fig. 4).

Activation of adenylate cyclase by ROS G unit and colchicine/wash supernatant saturates at 0.012 and 0.074 mg protein per ml respectively. Note that higher amounts of purified ROS G than endogenous G unit are required to reconstitute adenylate cyclase activity within the ESMF. This may be because ROS G is less able to insert successfully into the adenylate cyclase syncitium than is the native G unit. Nonetheless, it appears that the maximal Gpp(NH)p or NaF responsiveness conferred by added ROS G unit or colchicine/wash supernatant is limited by the Mn<sup>2+</sup>-determined catalytic activity (data not shown).

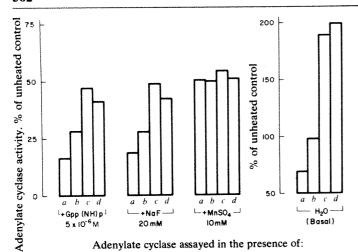


Fig. 4 Restoration of partially heat-inactivated adenylate cyclase activity with soluble synaptic membrane and rod outer segment components. ESMF were incubated at 42 °C for 30 min before assay for adenylate cyclase activity. Synaptic membrane supernatant (±colchicine) or ROS G unit (prepared as described in Fig. 3 legend) were added to the ESMF. The membranes were then assayed for adenylate cyclase (as described in Fig. 3 legend). Values are means of triplicate determinations from one of four similar experiments and are expressed as % of unheated controls. Unheated control values are: H<sub>2</sub>O, 43 pmol cyclic AMP per mg protein per min; Gpp(NH)p (5×10<sup>-6</sup> M), 290; NaF (20 mM), 231;

MgSO<sub>4</sub> (10 mM), 547. Supernatant additions: a, none; b, +control (no colchicine); c, +colchicine; d, +ROS G unit.

Figure 4 shows that Gpp(NH)p cannot reconstitute G unitmediated adenylate cyclase activation without added ROS G unit or synaptic membrane supernatant; increasing Gpp(NH)p (beyond  $5 \times 10^{-6}$  M) has no effect on adenylate cyclase activity. The supernatants, however, are capable of conferring additional guanyl nucleotide stimulation. ROS G unit or colchicine/wash supernatant charged with GDP do not increase basal adenylate cyclase activity in the differentially heat inactivated membranes (data not shown). When colchicine/wash supernatant or ROS G unit are heated (90 °C, 5 min) and added back to differentially heat-inactivated synaptic membranes, adenylate cyclase activities do not differ significantly from control (no supernatant added) values. Thus, these data indicate not only that free nucleotide is not responsible for restoration of adenylate cyclase activity, but that a heat-labile macromolecule with bound Gpp(NH)p is required.

The data presented here demonstrate that both free fatty acids and microtubule-disrupting agents enhance activation of rat synaptic membrane adenylate cyclase. Membrane-fluidizing lipids<sup>2,19</sup> or the local anaesthetic prilocaine<sup>3</sup>, and colchicine or are reported to activate adenylate cyclase in a vinblastine<sup>6-9</sup> number of cellular systems, and this activation seems to be distal to the hormone receptors<sup>2,3,8</sup>. The interaction of vinblastine at the G unit has been suggested to explain the biphasic effects of that compound in rat liver plasma membranes<sup>20</sup>. Our data suggest that the locus of the fatty acid and microtubule disruptor stimulation of synaptic membrane cyclase is at the G unit, as manganese response is not affected by these agents.

It is possible that the colchicine-mediated loss of Gpp(NH)p or fluoride responsiveness is due to a release of the G unit from the membrane. The ability to add back the same amount of G unit activity with colchicine/wash supernatant as with photoreceptor G unit supports this idea.

Most attempts to isolate the G unit have used detergents<sup>12</sup>. However, low salt washes can dislodge some G unit from the pigeon erythrocyte membrane<sup>21</sup>, there may be a soluble or a loosely attached G unit in liver cells<sup>22</sup> and previous experiments have observed a soluble factor which augments GTP or hormone sensitivity in liver plasma membranes23. The data presented here indicate that a subset of (though not necessarily all) G units may have a colchicine sensitive attachment to the synaptic plasma membrane.

The facilitation of G unit-mediated adenylate cyclase activity with either free fatty acids or microtubule-disrupting agents (without washing) suggests that the ability of the G unit to diffuse laterally in the membrane is a limiting factor in cyclase activation; that is, agents known to increase freedom of protein mobility in the membrane consequently increase cyclase activation. This effect may occur as a result of an increase in whole membrane fluidity or as a local effect at the site of G unit anchoring. Linoleic and cis-vaccenic (but not stearic) acids increased adenylate cyclase activity in these studies. The two former compounds (but not the latter) have recently been demonstrated to block lymphocyte capping in a manner similar to colchicine. In this sense, the fatty acids and microtubule disruptors might release constraints and enhance membrane protein mobility within localized domains5. A localized increase in membrane fluidity has similarly been proposed to explain the prilocaine augmentation of fluoride activated hepatic adenylate cyclase3

Loss of G unit activity mediated by colchicine or vinblastine but not fatty acids may be explained if one hypothesizes a microtubule (or membrane tubulin<sup>24</sup>) attachment site. We propose that fatty acids and microtubule-disrupting agents activate adenylate cyclase by different mechanisms. cis-Unsaturated fatty acids may increase membrane protein mobility, increasing the probability of effective G unit interaction with a catalytic moiety. Colchicine or vinblastine, however, may free the G unit from an attachment site, thus facilitating G unit-catalytic moiety interaction (and loss of G unit after washing). As not all the G unit activity can be eluted by colchicine, even after repetitive washing, it is likely that not all G units have a microtubule or tubulin attachment site. These data support a possible cytoskeletal-adenylate cyclase interaction and suggest additional possibilities for the control of adenylate cyclase activity.

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### A single amino acid change in IFN- $\beta_1$ abolishes its antiviral activity

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The cloning and expression of human fibroblast interferon (IFN- $\beta_1$ ) cDNA sequences in Escherichia coli have been reported by several groups 1-3; this will allow extensive study of its biological and physical properties. Aside from the cloning and expression of interesting and useful cDNA sequences, recombinant DNA techniques make possible the in vitro construction of gene sequences encoding novel polypeptides which do not occur naturally 4,8, which is difficult to achieve by direct chemical modification of proteins. We describe here the isolation of an IFN- $\beta_1$  cDNA sequence encoding the 117 Cterminal amino acids of a variant IFN- $\beta_1$  (Cys  $\rightarrow$  Tyr at amino acid position 141) and in vitro recombination with sequences coding for the N-terminal amino acids of IFN- $\beta_1$  to form a DNA sequence encoding a mature variant IFN- $\beta_1$  polypeptide, and its expression in E. coli. We show that the replacement of Cys at position 141 with Tyr inactivates antiviral activity in a cytopathic effect (CPE) inhibition assay and that the variant protein does not compete with normal fibroblast interferon for reaction with heterospecific antibodies to IFN- $\beta_1$ . These effects are attributed to an alteration in protein structure of IFN- $\beta_1^{Cyn-Tyn}$  which result from its inability to form an essential disulphide bond.

In our efforts to isolate a recombinant plasmid containing the cDNA sequences encoding human fibroblast interferon (IFN- $\beta_1$ ), several partial-length cDNA clones were obtained<sup>3</sup>. Among these is pF526, which is ~640 base pairs (bp) long and contains the poly(A) sequence, the 3'-untranslated region and about two-thirds of the coding region for the mature IFN- $\beta_1$  protein. DNA sequence analysis showed two changes from the published sequence of IFN- $\beta_1$  (Fig. 1; refs 1-3). The first involves the deletion of nucleotides 762-764 (GTC) immediately preceding the poly(A) in the 3'-noncoding region and may be due to normal variation in processing/polyadenylation of the primary transcript. The second change is a  $G \rightarrow A$  transition at position 485 of the published sequence, which lies within the coding region for the mature polypeptide and results in the replacement of a cysteine (TGT) codon with a tyrosine (TAT) codon. Because all the leukocyte interferons (IFN- $\alpha$ ) described to date<sup>6</sup> have a cysteine residue which can be aligned with the cysteine at position 141 of IFN- $\beta_1$  and as this cysteine is involved in disulphide bond formation in the IFN- $\alpha$  family<sup>7</sup>, it seemed likely that IFN- $\beta_1^{C_{T}\to T_{T}}$  might be a useful variant for investigating the structure of the IFN- $\beta_1$  molecule in relation to its antiviral activity.

As the cDNA insert of pF526 comprises only a partial copy of the IFN- $\beta_1$  mRNA and encodes only the 117 C-terminal amino acids of the mature polypeptide (Fig. 1), it was necessary to prepare a hybrid gene containing DNA sequences for the N-terminal 56 amino acids of the mature polypeptide and pF526 sequences encoding the remaining 110 acids. As shown in Fig. 2, pFIF  $tp^3$  69 (ref. 3) is a pBR322 derivative which carries three tp promoter fragments all oriented so that transcription occurs towards the cDNA insert encoding mature IFN- $\beta_1$ . The IFN- $\beta_1$  cDNA expression insert is bounded by XbaI (5') and BgIII (3') restriction endonuclease sites<sup>3</sup>. We removed the XbaI-BgIII insert from pFIF  $tp^3$  69, discarded the 3' HgaI-BgIII gene fragment and replaced it with the corresponding sequences from the variant. The recombined sequences were inserted into the original expression vehicle containing three promoters to

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Fig. 1 The normal and variant IFN- $\beta_1$  cDNA sequences. Numbers above each line refer to nucleotide position, and numbers below refer to amino acid residue (S, signal peptide). The variant cDNA clone is underlined. The G $\rightarrow$ A transition is indicated by an arrow at position 485. The three nucleotides deleted in pF526 are indicated by asterisks above the normal sequence at positions 762–764. These cDNAs were cloned and isolated as described elsewhere<sup>3</sup>.

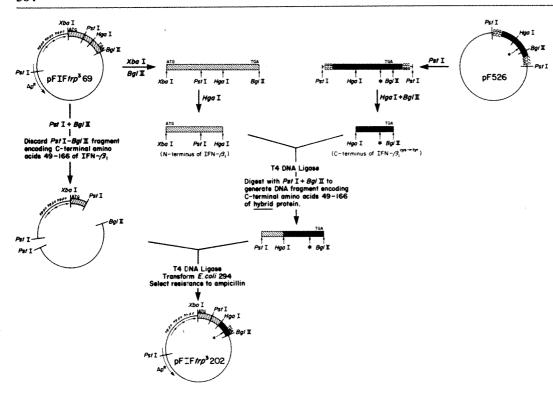
achieve maximal expression (Fig. 2). Restriction endonuclease and DNA sequence analysis of the new expression plasmid, pFIF  $trp^3$  202, showed that the promoters, the ribosome binding site and the entire hybrid coding region gene were arranged correctly (results not shown).

The interferon activity present in lysates of E. coli 294 containing the wild-type (pFIF trp<sup>3</sup> 69) and variant (pFIF trp<sup>3</sup> 202) expression plasmids as measured in a CPE inhibition assay<sup>3</sup>, was  $1.6 \times 10^4$  U ml<sup>-1</sup> for  $294/pFIF trp^3$  69 extracts but <21 U ml<sup>-1</sup> for extracts prepared from 294/pFIF trp<sup>3</sup> 202 (Table 1). Because the Cys → Tyr alteration may have affected the folding of the polypeptide, rendering it more labile to E. coll proteases, it was necessary to show that a stable protein with some of the properties of IFN- $\beta_1$  was detectable in extracts prepared from E. coll. As biosynthetic fibroblast interferon comprises <0.1% of E. coli protein it was not possible to show that equivalent amounts of both proteins were present in the extracts used for the initial assays shown in Table 1. To confirm that IFN- $\beta_1^{\text{Cyp-Tyr}}$  was inactive we synthesized both proteins in a coupled in vitro transcription-translation system derived from E. coli PR13 (ref. 8). Both pFIF trp<sup>3</sup> 69 and pFIF trp<sup>3</sup> 202 were used to programme the in vitro system and the products were analysed by SDS-polyacrylamide gel electrophoresis (Fig. 3) and tested for antiviral activity. Equivalent amounts of both wild-type and variant proteins were synthesized in vitro (Fig. 3).

Table 1 Antiviral activities of wild-type and variant IFN- $\beta_1$ 

Sample	Dilution of lysate	Interferon activity (U ml <sup>-1</sup> )
FIFN trp <sup>3</sup> 69 FIFN trp <sup>3</sup> 69 FIFN trp <sup>3</sup> 202 FIFN trp <sup>3</sup> 202 FIFN trp <sup>3</sup> 202 FIFN trp <sup>3</sup> 202	10 <sup>-1</sup> 10 <sup>-2</sup> - 10 <sup>-1</sup> 10 <sup>-2</sup>	1.6×10 <sup>4</sup> 1.3×10 <sup>3</sup> 1.7×10 <sup>2</sup> <21 <21 <21

Lysates were prepared and assayed by CPE inhibition<sup>3</sup>. Different dilutions are shown to indicate the range of variability in the assay.



2 Construction of IFN- $\beta_1$ expression plasmid. The starting materials were constructed as described elsewhere<sup>3</sup>. All enzymes were from New England Biolabs and digestions were done in 60 mM Tris pH 7.5. 6 mM MgCl<sub>2</sub>, 60 mM NaCl and 6 mM 2mercaptoethanol at 37 °C. Ligations were done overnight at 12°C in 60 mM Tris pH 7.5, 3 mM MgCl<sub>2</sub> and 10 mM dithiothreitol. Transformations done as described pre-viously<sup>3</sup>. Transformed Transformed cells were selected on LBagar plates containing 20 µg ml<sup>-1</sup> ampicillin to ensure that only clones containing plasmids with the correct structure were recovered. Arrows within plasmids represent trp promoter fragments and their direction of transcription. Cross-hatched blocks represent wildtype coding sequences and shaded blocks variant coding sequences.

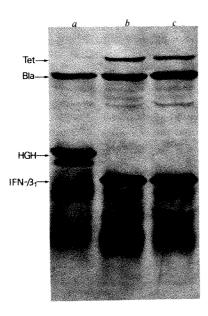


Fig. 3 In vitro synthesis of normal and variant IFN- $\beta_1$ . Plasmids encoding human growth hormone (a), IFN- $\beta_1^{\text{Cys-Tyr}}$  (b) and wild-type IFN- $\beta_1$  (c) were used to programme a coupled in vitro transcription-translation system. The top two bands in each case are the plasmid-encoded protein for tetracycline resistance (Tet; molecular weight  $(M_r)$  36,000) and  $\beta$ -lactamase (Bla; $M_r$  32,000). The lower band in a is human growth hormone (HGH;  $M_r$  22,000) encoded by a plasmid similar in structure to the expression plasmids encoding the variant and wild-type IFN- $\beta_1$  ( $M_r$  20,000). The in vitro system used was that described in ref. 8. Plasmid DNA (5  $\mu$ g) was added to a total reaction volume of 25  $\mu$ l. 5  $\mu$ l of each reaction mixture was analysed by electrophoresis through a 15% SDS-polyacrylamide gel<sup>12</sup> and subsequently fluorographed as described by Bonner and Laskey<sup>13</sup>. 10  $\mu$ l of each reaction mixture was used to determine antiviral activity in a CPE inhibition assay<sup>3</sup>.

When aliquots of the *in vitro* reactions were used in a CPE inhibition assay, extract programmed by pFIF  $trp^3$  69 yielded  $3 \times 10^3$  U ml<sup>-1</sup> IFN- $\beta_1$  antiviral activity, whereas pFIF  $trp^3$  202 extracts had no detectable activity.

Although the variant fibroblast interferon, IFN- $\beta_1^{\text{Cys} \rightarrow \text{Tyr}}$  shows no antiviral activity it might still compete with the other form of IFN- $\beta_1$  in binding antibodies against fibroblast interferon. Incubation of IFN- $\beta_1$  with up to a 1,000-fold excess of IFN- $\beta_1^{\text{Cys} \rightarrow \text{Tyr}}$  and sufficient heterospecific rabbit anti-fibroblast interferon antibodies (obtained from the Antiviral Substances Program of the National Institutes of Allergy and Infectious Diseases) to neutralize the wild-type IFN- $\beta_1$  only, no antiviral activity was observed (data not shown). These results indicate that the amino acid change in IFN- $\beta_1^{\text{Cys} \rightarrow \text{Tyr}}$  disrupts the structure of the molecule sufficiently to prevent it from binding antibody specific for that part of the protein essential for induction of antiviral activity in target cells.

The Cys  $\rightarrow$  Tyr change introduced in the fibroblast interferon variant described here could disrupt the function of the molecule in a number of ways, for example, by altering the strength of intramolecular hydrogen bonding or hydrophobic interactions. However, because preincubation of IFN- $\beta_1$  extracts with dithiothreitol completely abolishes antiviral activity in a CPE inhibition assay (H.M.S., unpublished observations), it is likely that the disulphide bond between Cys 31 and Cys 141 is essential to maintain the structure of IFN- $\beta_1$  required for antiviral activity. Our data therefore suggest that the altered structure of IFN- $\beta_1$  revents it from binding to cell membrane receptors, an interaction which mediates the antiviral activity of interferon.

Variant interferons have been found in the leukocyte interferon family, notably two forms of LeIFN H, which could represent allelic forms<sup>6</sup>. Between the IFN- $\alpha$  species A and D there are a total of 29 amino acid differences<sup>6</sup>. Of these, three occur within the region which is most conserved among the IFN- $\alpha$  species (between amino acids 115 and 151; refs 6, 10). Therefore, some amino acid sequence changes within this conserved region are tolerated, although the cysteine at position 139 (141 of IFN- $\beta_1$ ) may be essential for antiviral activity

(R. Wetzel, personal communication). Because the 5'-terminal sequences of IFN-β<sub>1</sub><sup>Cys - Tyr</sup> are unknown it is uncertain whether it is analogous to the variants of the leukocyte interferon family. It is possible that the  $G \rightarrow A$  transition at nucleotide 486 (Fig. 1) is the result of a reverse transcription error and does not occur naturally. Of four partial-length cDNA clones that were obtained from the human fibroblast cell line GM-2504A (ref. 3), only pF526 had the Cys → Tyr change at amino acid 141 and the deletion of nucleotides 762-764 immediately preceding the polyadenylation site. In any case it is possible that the variant IFN- $\beta_1$  mRNA identified by cDNA cloning is not normally translated, or if it is, it could be biologically active by virtue of some compensating changes in the unknown segment of the gene. The variant IFN- $\beta_1$  gene cannot correspond to the proposed second fibroblast interferon gene (IFN- $\beta_2$ ) because IFN- $\beta_2$  sequences show little or no cross-hybridization with IFN- $\beta_1$ (ref. 11). Cell membrane binding studies are in progress to determined directly whether IFN- $\beta_1^{\text{Cys} \rightarrow \text{Tyr}}$  is unable to bind cell receptors.

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#### Non-muscle $\alpha$ -actinins are calcium-sensitive actin-binding proteins

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Many actin-binding proteins have been purified from different cell types, and a number of them share certain features: they are affected by calcium in their interaction with actin and have similar subunit molecular weights (~100,000) on SDS-polyacrylamide gels. These proteins have been given various names, including gelsolin<sup>1-3</sup>, villin<sup>4,5</sup>, actinogelin<sup>6,7</sup>, *Dictyostelium* 95K protein<sup>8,9</sup>, platelet 95K protein<sup>10</sup> and *Acanthamoeba* 85K protein<sup>11</sup>. The actin-binding protein α-actinin has received little attention in this analysis, even though it has a subunit molecular weight in this range ( $\sim 100,000$ ) and has been shown immunologically to exist in non-muscle cells<sup>12,13</sup>. Here we report that  $\alpha$ -actinin purified from HeLa cells is inhibited from crosslinking actin filaments by micromolar free calcium. In many properties it closely resembles actinogelin, Dictyostelium 95K protein and Acanthamoeba 85K protein, but it appears distinct from proteins such as gelsolin and villin which, in the presence of calcium, fragment actin filaments into short oligomers<sup>3</sup> conclude that in this molecular weight range there are at least two classes of non-muscle actin-binding protein: one corresponding to non-muscle  $\alpha$ -actinins and the other to proteins such as gelsolin and villin.

α-Actinin has been purified from cultured HeLa cells using a modification of the procedure we developed for the purification of  $\alpha$ -actinin from smooth muscle<sup>14</sup>. Chromatography of this  $\alpha$ -actinin on hydroxyapatite resulted in the separation of two

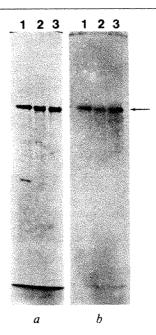


Fig. 1 SDS gel analysis of HeLa cell  $\alpha$ -actinins and  $\alpha$ -actinins from chicken gizzard smooth muscle. a, A 10% polyacrylamide SDS gel stained with Coomassie blue on which were electrophoresed 4µg of the following proteins: track 1, smooth muscle α-actinin; track 2, HeLa α-actinin I; track 3, HeLa α-actinin II. The slight difference in molecular weight between the two forms of HeLa cell α-actinin is not detectable at this level of protein loading on the gel. b, An autoradiograph of the same gel slice after staining the gel with antibodies against beef cardiac  $\alpha$ -actinin followed by a second radio-iodinated antibody directed against the first antibody. Note that the antibody against  $\alpha$ -actinin labels all three proteins. This antibody has been characterized previously<sup>18</sup> and only a tight doublet of bands corresponding to the two  $\alpha$ -actinins is labelled when a gel of total HeLa cell proteins is stained with this antibody <sup>18</sup>. The gel staining with antibody was performed as described elsewhere <sup>19</sup>. SDS-gel electrophoresis was performed using Laemmli's buffer conditions <sup>20</sup>.

forms with slightly different subunit molecular weights on SDS gels (~102,000 and 100,000). The detailed description of the purification and characterization of these two forms of HeLa α-actinin will be described elsewhere (J.R.F. and K.B., in preparation). Figure 1 shows SDS-polyacrylamide electrophoretic gels of the two forms (referred to here as HeLa  $\alpha$ -actinin I and HeLa  $\alpha$ -actinin II) next to a sample of smooth muscle  $\alpha$ -actinin (Fig. 1a). An autoradiograph of the same gel is also shown (Fig. 1b) after incubation with antibody against muscle α-actinin followed by incubation with a second radioiodinated antibody directed against the first antibody. This indicates that both HeLa proteins cross-react immunologically with muscle  $\alpha$ -actinin. In addition to their similar purification properties, subunit molecular weights on SDS gels and crossreaction with antibodies to muscle  $\alpha$ -actinin, these two HeLa proteins have been identified as  $\alpha$ -actinin by several criteria that will be described in detail elsewhere (J.R.F. and K.B., in preparation). Briefly, both native proteins are dimers, with Stokes' radii and s values close to those of muscle  $\alpha$ -actinin. Their native peptide maps and amino acid composition are similar to but distinct from those of muscle  $\alpha$ -actinin. Both proteins bind to F-actin and this binding is diminished by tropomyosin at 37 °C. as is found with muscle  $\alpha$ -actinin. Unlike muscle  $\alpha$ -actinin, however, the binding to F-actin is markedly inhibited by low free calcium concentrations (Figs 2, 3). In low free calcium ( $<10^{-7}$  M), the two non-muscle  $\alpha$ -actinins

are potent actin gelation factors. The concentration of the  $\alpha$ -actinins that will cause gelation of F-actin (as determined by the failure of a stainless-steel ball to fall in the failing ball viscometer) varies between experiments and is dependent on conditions such as actin concentration, temperature and age of  $\alpha$ -actinin preparations. Typically, with F-actin at

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Table 1	Classification of calcium-sensitive actin-binding proteins				
I	Non-muscle α-actinin class				
	HeLa α-actinins				
	Platelet 105K protein				
	Actinogelin				
	Dictyostelium 95K protein				
	Acanthamoeba 85K protein				
II	Gelsolin/villin class				
	Gelsolin				
	Villin				

The class I proteins cross-link F-actin at low calcium concentrations but are inhibited from cross-linking at high calcium concentrations. They appear to be rod-shaped proteins, and in some cases have been shown to be dimeric. The class II proteins are globular, monomeric proteins that in the presence of calcium fragment actin filaments into short oligomers.

Platelet 95K protein

 $0.2~{\rm mg~ml}^{-1}$  gelation is seen at an  $\alpha$ -actinin: actin molar ratio of 1:100-1:200, but at  $0.4~{\rm mg~ml}^{-1}$  F-actin gelation has been seen at a molar ratio of about 1:400 and this ratio will decrease still further as the actin concentration is increased.

The effect of different free calcium concentrations on the viscosity of mixtures of actin and  $\alpha$ -actinin is shown in Fig. 3. Smooth muscle  $\alpha$ -actinin is essentially unaffected by calcium concentrations in the range examined here. With the two forms of HeLa  $\alpha$ -actinin a sharp fall in the viscosity occurs when the free calcium concentration exceeds  $10^{-7}$  M. Note, however, that the viscosity only falls to the background level of the F-actin on its own, indicating that the cross-linking by  $\alpha$ -actinin has been inhibited. On the other hand, with a protein such as gelsolin in the presence of micromolar calcium and F-actin, the viscosity of the mixture decreases below the level of the F-actin control due

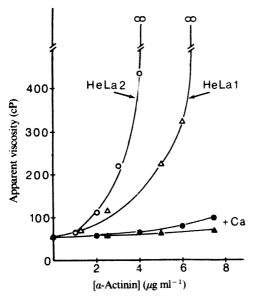


Fig. 2 Effect of varying concentration of HeLa  $\alpha$ -actinin on the viscosity of F-actin. The two forms of HeLa  $\alpha$ -actinin were added to preformed F-actin  $(0.2 \text{ mg mi}^{-1})$  at low  $(10^{-7} \text{ M})$  and high  $(10^{-4} \text{ M})$  free calcium.  $\triangle$ , HeLa  $\alpha$ -actinin I at low free calcium;  $\triangle$ , HeLa  $\alpha$ -actinin II at low free calcium;  $\bigcirc$ , HeLa  $\alpha$ -actinin II at high free calcium;  $\bigcirc$ , HeLa  $\alpha$ -actinin II at high free calcium. The final ionic conditions were 40 mM KCl, 1 mM MgCl<sub>2</sub>, 0.1 mM ATP, 0.275 mM EGTA, 0.1%  $\beta$ -mercaptoethanol, 10 mM PIPES,  $\beta$ H 7.0, for those measurements at  $<10^{-7}$  M free calcium, or for those measurements at  $10^{-4}$  M free calcium the same ionic conditions were used except that the EGTA was replaced by 0.1 mM CaCl<sub>2</sub>. Apparent viscosity was measured using the low-shear, falling ball viscometer  $^{21}$  at 21 °C, 2 h after mixing the samples. Note that in the presence of  $10^{-4}$  M calcium the viscosity remained essentially at the background level generated by F-actin alone (apparent viscosity = 56 cP), whereas below  $10^{-7}$  M calcium gelation was induced by  $\sim 5 \text{ μg ml}^{-1}$  of HeLa  $\alpha$ -actinin II or  $\sim 1.5 \text{ μg ml}^{-1}$  of HeLa  $\alpha$ -actinin II.

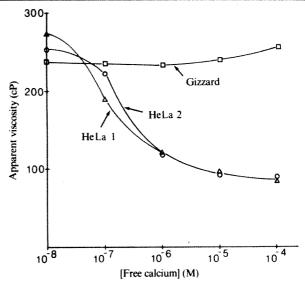


Fig. 3 Effect of varying free calcium concentration on the viscosity generated by mixing purified HeLa cell or chicken gizzard smooth muscle  $\alpha$ -actinins with F-actin. HeLa  $\alpha$ -actinin I  $(\triangle)$  was used at a final concentration of 5  $\mu$ g ml<sup>-1</sup>, HeLa  $\alpha$ -actinin II  $(\bigcirc)$ was used at 3.5  $\mu$ g ml<sup>-1</sup>, chicken gizzard smooth muscle  $\alpha$ -actinin ( $\square$ ) was used at  $\sim 10 \ \mu$ g ml<sup>-1</sup>. The  $\alpha$ -actinins were added to preformed F-actin, used at a final concentration of 0.2 mg ml The final ionic conditions were identical to those in Fig. 2 except the free calcium concentrations were generated using a Ca-EGTA buffer in which the EGTA concentration was maintained at 0.275 mM, apart from measurements of the values at 10 calcium, from which EGTA was omitted and replaced by 0.1 mM CaCl<sub>2</sub>. Free calcium concentrations were calculated using an apparent association constant for EGTA and calcium of log 6.68 (ref. 22). Any effect of 0.1 mM ATP was ignored in this calculation. Viscosity was measured using a falling ball viscometer at 23 °C 3 h after mixing the samples. The apparent viscosity of the F-actin without α-actinin was 78 cP and was unaffected by the different calcium concentrations. Note that in the higher free calcium concentrations the viscosity of the samples with either of the two HeLa  $\alpha$  -actinins fell to a level approaching that of the F-actin alone but did not fall below this value.

to the severing of the actin filaments to short oligomers by this protein<sup>3</sup>.

The two forms of  $\alpha$ -actinin from HeLa cells are very similar to the muscle  $\alpha$ -actining by all criteria so far examined, with the exception of this calcium-inhibited interaction with actin. In collaboration with Rosenberg and Stracher, we have recently demonstrated that a platelet protein of molecular weight 105,000 (105K protein) that is calcium sensitive in its binding to actin is the platelet  $\alpha$ -actinin<sup>15</sup>. The calcium sensitivity of both HeLa and platelet  $\alpha$ -actinins suggests that this is a general property of non-muscle  $\alpha$ -actinins. On examining the other actin-binding proteins in this molecular weight range, we note that several closely resemble the HeLa  $\alpha$ -actinins in response to calcium and in many of their other properties. Actinogelin closely resembles muscle  $\alpha$ -actinin in all physical properties examined so far (A. Asano, personal communication). Acanthamoeba 85K protein resembles a-actinin in being a rodshaped protein with a Stokes' radius of about 85 Å (ref. 11). Dictyostelium 95K protein is also rod-shaped and preliminary data indicate that, like a-actinin, it is dimeric (D. L. Taylor, personal communication). All these proteins cross-link F-actin in a manner that is inhibited by calcium<sup>7,8,11</sup>. On the other hand, proteins such as gelsolin and villin seem to be quite different, being globular, monomeric proteins that also bind to actin but, in the presence of calcium, fragment actin filaments to shorter oligomers<sup>2,3,5</sup>. We have, therefore, grouped these actin-binding proteins in Table 1 into two distinct classes: an  $\alpha$ -actinin class which would include the HeLa a-actinins of the present study, the platelet 105K protein, actinogelin, Dictyostelium 95K protein and Acanthamoeba 85K protein; and a second class that would include gelsolin and villin. As T. P. Stossel and his

colleagues have identified gelsolin-like proteins immunologically in a wide variety of different vertebrate cell types (personal communication), it may be that most higher eukaryotic cells contain representatives of both classes of protein. So far, a protein similar to gelsolin has not been identified in this molecular weight range in lower eukaryotes, and the equivalent function may be served by a protein of lower molecular weight. Such a protein, called fragmin, with an apparent molecular weight of 45,000, has been purified from Physarum 16,17, and a low molecular weight protein with similar properties has also been identified in Dictyostelium (J. Spudich, personal communication).

The difference between non-muscle  $\alpha$ -actinins and muscle α-actinins in their calcium-sensitive interaction with actin may reflect the different requirements of the muscle and non-muscle contractile systems. With muscle, a comparatively stable actin

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lattice is required for efficient repeated contraction, but with most non-muscle cells movement is generally accompanied by a change of shape. It seems necessary that the cell's forcegenerating apparatus should be able to be disassembled and reorganized rapidly for movement to continue. Given this requirement, it may well be an advantage for a non-muscle cell to have an  $\alpha$ -actinin that will respond to a calcium flux. In the absence of calcium, non-muscle α-actinin would cross-link actin filaments to form a stable network, but in the presence of calcium it would release the actin filaments and so permit their rapid reorganization.

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#### Myosin filaments have non-phosphorylated light chains in relaxed smooth muscle

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The provocative hypothesis that myosin in resting smooth muscle is in a soluble form and assembles into filaments only at the time of contraction1,2 has been disproved with the demonstration of regular arrays of myosin filaments in a variety of smooth muscles, including those shown to have been relaxed before and during fixation3-7. Nevertheless, following the observations of Suzuki et al.8 on the effect of light-chain phosphorylation on smooth muscle myosin assembly in vitro, this hypothesis has been revived with the speculation that relaxed smooth muscle contains non-phosphorylated, non-filamentous myosin that assembles into filaments only after the muscles are stimulated and myosin is phosphorylated9. Here we have used rapid freezing techniques to avoid concerns about the possible effects of fixation on the in vivo form of myosin10 and demonstrate the existence of a myosin filament lattice in relaxed vascular smooth muscles in which we also show myosin light chain phosphorylation to be minimal (<5%).

Longitudinal strips of rabbit portal anterior mesenteric vein were attached to force transducers, stretched to physiological (1.75× slack) length, mounted in muscle baths at 37 °C in oxygenated Krebs solution and equilibrated for ~1 h. Some muscles were exposed for 1.5 min to 0.2 µg ml<sup>-1</sup> isoprenaline which is known to hyperpolarize and relax smooth muscle 11. The muscle bath was quickly lowered and a beaker of supercooled Freon 22 (ref. 12) was shot up to the muscle. The moment of impact was seen on the tension record that showed that the

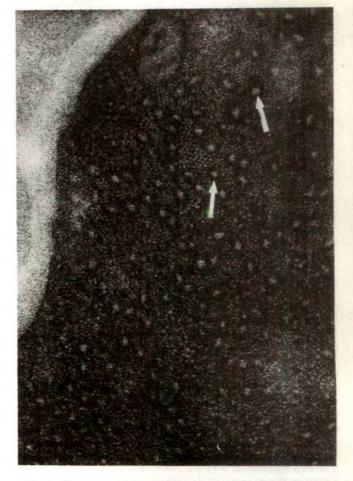


Fig. 1 Transverse section of a portion of an isoprenaline-relaxed PAMV smooth muscle cell, rapidly frozen and freeze substituted in acetone for 3 days at -80 °C. Osmium crystals were added on warming and en bloc staining was done with uranyl acetate in methanol, which resulted in a negative staining effect in some of the outer cells. Arrows indicate myosin filaments surrounded by actin (thin) filaments. ×138,000.

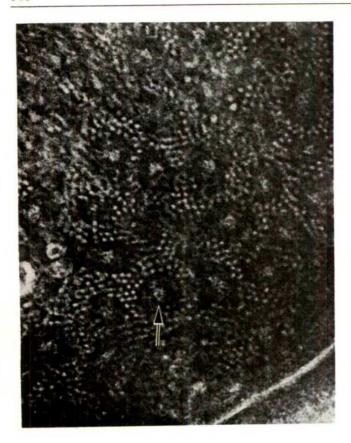


Fig. 2 Transverse cryosection of a rapidly frozen, briefly (15 min) glutaraldehyde-fixed muscle. The section was picked up on a drop of frozen sucrose in the cryo chamber, melted in a dry atmosphere and negatively stained with 1% ammonium molybdate. Myosin filaments (arrow) are surrounded by rosettes of actin filaments. ×200,000.

muscle remained relaxed. The strips were processed by freeze substitution  $^{13.14}$  for ultrastructural studies or analysed for the degree of myosin light chain phosphorylation. The phosphorylated and unphosphorylated forms of the 20,000 molecular weight chain (LC<sub>20</sub>) were separated by isoelectric focusing  $^{15}$  in urea–polyacrylamide gels followed by electrophoresis in SDS-containing slab gels in the second dimension  $^{16-18}$ .

In relaxed, freeze-substituted muscles (Fig. 1), myosin filaments were consistently observed in the well frozen surface cells (nine muscles). The number and distribution of myosin filaments were similar in rapidly frozen and chemically fixed muscles<sup>3,19</sup> (Figs 2, 3). The demonstration of thick filaments in the rapidly frozen freeze-substituted muscles renders highly unlikely the implication<sup>10</sup> that the use of glutaraldehyde fixation before freezing (Fig. 2) induces the formation of an array of myosin filaments.

Regular arrays of actin filaments were more difficult to maintain in the rapidly frozen muscles, due to disorder caused by minute ice crystals. Therefore, three muscles were preincubated in a cryoprotectant—10% bovine serum albumin, 10% ethylene glycol or 10% dimethyl sulphoxide in Krebs solution—for 5 min before the addition of isoprenaline. Ethylene glycol and dimethyl sulphoxide abolished the spontaneous contractions. Ethylene glycol provided the greatest depth of cryoprotection and preserved the order of the actin filaments (Fig. 4). In these preparations, the ratio of actin to myosin filaments was 12:1, similar to the ratios found in conventionally fixed muscles<sup>3,19</sup>.

The average  $LC_{20}$  phosphorylation in portal veins relaxed with isoprenaline (six animals) was less than 5% (Fig. 5a). An

additional three relaxed portal veins which were cryoprotected before freezing (Fig. 4) showed similarly low levels of LC20 phosphorylation as untreated resting muscles. In contrast to the relaxed muscles, significant levels of LC20 phosphorylation, ranging over 23-73%, were measured in muscles frozen 2-15 s after stimulation with a high K+ depolarizing solution (Fig. 5b, c), with an average time-to-peak tension after activation of 12 s. The absence of any significant phosphatase activity during extraction of contractile proteins was demonstrated in control experiments using purified gizzard actomyosin in which LC20 was ~80% phosphorylated (as shown by 32P incorporation): phosphorylated actomyosin was (1) ground up with frozen taenia coli before extraction, (2) extracted separately or (3) ground up with frozen taenia coli and incubated at room temperature for 20 min before extraction. In (1) and (2) at most 4% of the 32P label was released during the normal extraction procedure, while in case (3) almost all the labelled phosphate was released, as also demonstrated by the absence of phosphorylated LC20 in the two-dimensional gels. Other control experiments showed that there was no change in LC20 phosphorylation when the frozen homogenates were incubated for up to 4 h at room temperature in the solution (9.0 M urea, 5% mercaptoethanol) used for isoelectric focusing. These controls and the clear demonstration of phosphorylation of light chains in contracted muscle indicate that the extraction procedure and isoelectric focusing followed by SDS-gel electrophoresis provide an accurate estimate of the degree of LC20 phosphorylation.

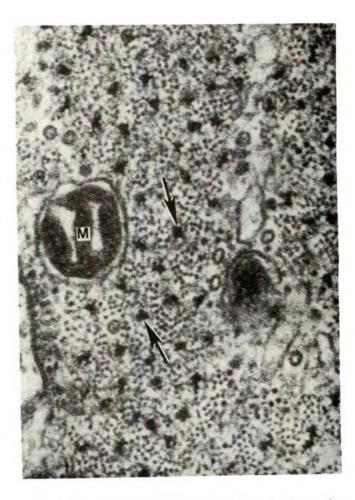


Fig. 3 Transverse section of an isoprenaline-relaxed smooth muscle, fixed in 2% glutaraldehyde followed by 2% tannic acid before post-fixation in osmium and *en bloc* staining with uranyl acetate. Arrows indicate myosin filaments; M, mitochondrion. ×148,000.

That most of the myosin in mature smooth muscle is in filamentous form is also supported by the close agreement between estimates of myosin content obtained by SDS gels<sup>7,20-22</sup> and by quantitative electron microscopy3,19. The same actin: myosin ratios in rabbit portal anterior mesenteric vein were obtained when measured by either SDS-gel electrophoresis or quantitative electron microscopy (P. F. Berner, A.V.S., A.P.S. and H. Holtzer, unpublished observations). We also note that short filaments ( $\sim 0.5 \mu m$ ) are produced by phosphorylation of myosin in vitro, unlike the 2.2-µm long filaments found in smooth muscle in situ 19

Thus, there is compelling evidence that myosin in resting smooth muscle is predominantly filamentous, even when the light chains are unphosphorylated, although we do not wish to extrapolate our findings to non-muscle cells. The rapid freezing techniques (refs 12, 23-25 and present study) that circumvent the objections that chemical fixation and associated changes in ionic strength, cation and nucleotide concentrations may induce the formation of myosin filaments also seem to be the most direct method of exploring the in situ state of myosin in nonmuscle cells.

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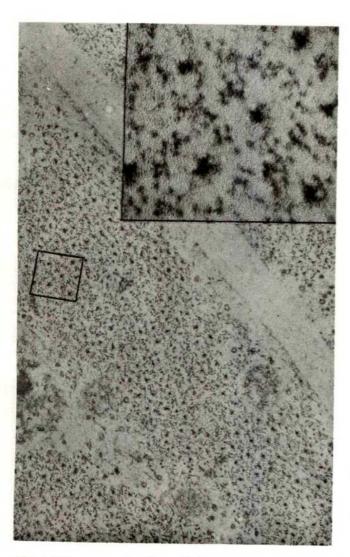
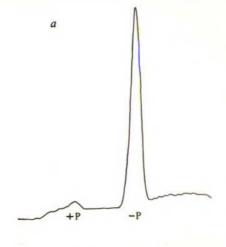
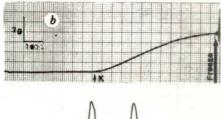


Fig. 4 Transverse section of a rapidly frozen isprenaline-relaxed smooth muscle which has been cryoprotected with 10% ethylene glycol. ×60,000. Inset: regular arrays of thick filaments occur which are surrounded by actin filaments shown at higher magnification (×330,000). LC20 phosphorylation in this muscle





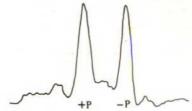


Fig. 5 a, Densitometer trace of a gel of an isoprenaline-relaxed muscle showing less than 5% phosphorylation of the LC20. b, Tension trace of a portal vein exposed to a high K+ depolarizing solution. The muscle was rapidly frozen at 6 s (arrow) by shooting a beaker of supercooled Freon 22 up to the muscle. c, Densitometer trace of the LC20 myosin light chains in an aliquot of the same, contracted muscle, showing that 50% of the LC20s are in the phosphorylated form. For the phosphorylation studies, the frozen muscles were pulverized with 0.5 M HClO4 at liquid N2 temperature. After thawing, the protein precipitate was dissolved in 9 M urea and 5% mercaptoethanol.

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# Stress does not alter the conformation of a domain of the myosin cross-bridge in rigor muscle fibres

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The force of muscle contraction is thought to be generated by a change in the effective angle of a myosin cross-bridge while it is attached to an actin filament. Thus studies of the relationship between this conformation and force provide insight into the mechanism of contraction. Recently it has been shown¹ that paramagnetic probes can be attached selectively and rigidly to a reactive sulphydryl on the myosin cross-bridge and that their angular distribution can be measured with great accuracy. In rigor skeletal fibres the probes are highly ordered relative to the fibre axis. Here I show that forces of up to 0.2 N mm⁻² cause no change in the angular distribution of the probes. Thus there is a domain of the cross-bridge whose conformation is not influenced by stress, and this result places restrictions on the location of elastic and force-generating elements within the cross-bridge.

The heads of the myosin molecule form bridges between the myosin and actin filaments. During contraction several changes in the conformation of these cross-bridges are thought to occur, including the stretching of an elastic element and a change in the configuration of some force-generating element resulting in a large (~45°) change in the effective angle of the cross-bridge<sup>2.3</sup>. The location of these elements within the cross-bridge is unknown; one possibility is that one or both may reside in the actin-myosin bond. By measuring the angle of one portion of myosin in a rigor fibre, that is, a fibre in the absence of ATP, we provide information on the location of these elements.

Various methods have been used to monitor the angle of the myosin heads in muscle fibres, including electron microscopy4, X-ray diffraction<sup>5</sup> and fluorescence spectroscopy<sup>6</sup>. Only two of these have been used to explore the relationship between force and cross-bridge orientation in rigor fibres. Naylor and Podolsky have shown that the intensity ratio of two equatorial reflections in the X-ray diffraction pattern did not change on application of a static strain, and dos Remedios et al. showed that the polarization of tryptophan fluorescence did not change when the rigor fibre was subjected to a periodic strain. For both these methods, the parameters observed reflected the conformation of the entire fibre, the X-ray being sensitive to the distribution of mass in the filament array and the fluorescence being derived from all tryptophans in the fibre. Thus, paramagnetic probes, which allow accurate and direct measurement of the angular conformation of one portion of the myosin molecule, provide quite different information from that obtained using these other methods.

Two probes were used having reactive groups that resembled iodoacetamide (IASL) and melaimide (MSL). Both labels have been shown to react selectively with a sulphydryl on the myosin head, designated  $SH_1$  (refs 2, 9). The hyperfine interaction between the unpaired electron and the nitrogen nucleus splits the spectrum of a nitroxide radical into three lines. Both the splitting between these lines and the g value depend on the angles between spin probe axes and the magnetic field of the spectrometer<sup>2,10</sup>. When the magnetic field is aligned along the fibre axis this effect allows measurement of the angle between this axis and the principle axis of the spin probe.

Figure 1 shows the spectra of fibres labelled with IASL. The fibres are in rigor at a sarcomere length of 2.2-2.4 µm where all the myosin heads are overlapped by thin filaments. These spectra are seen to consist essentially of three lines, indicating that most of the probes have the same approximate angle with respect to the fibre axis. It was shown previously<sup>2</sup> that such spectra are well described by assuming that the angular distribution of probes is gaussian, centred at 68° with a full width at half height of 15°. As shown in Fig. 1, a static stress applied to the fibres caused little alteration in their spectra. It was expected that stress would result in: (1) a shift in the average angle of the gaussian distribution: (2) a broader distribution and/or (3) that the cross-bridges would assume some new and radically different distribution. Any shift in the mean angle of the gaussian distribution would result in a shift in the splitting between the peaks. This splitting, indicated by arrows in Fig. 1, is shown in Fig. 2 for both labels as a function of the stress on the fibre. There was no change in the mean angle of either probe within

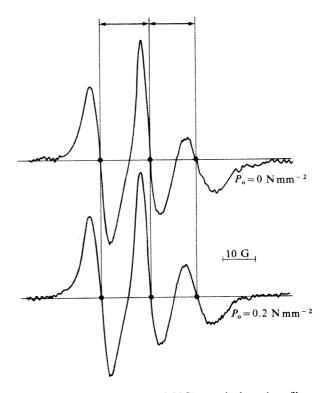


Fig. 1 Paramagnetic spectra of IASL attached to rigor fibres shown for two cases: above, tension = 0; below, tension =  $0.2 \text{ N mm}^{-2}$ . The fibres were labelled with 0.5 mM IASL for 1 h following procedures described elsewhere1. Several methods, including quantitation of peak heights in the spectrum and the decrease in the EDTA-ATPase of myosin extracted from the fibres, indicated that 70-100% of the myosin heads were labelled specifically on the reactive SH by this procedure. The rigor stiffness and the ability to generate isometric tension were both unchanged by labelling of the myosin heads. Spectra were obtained using a Varian E-3 spectrometer with a cavity modified to permit simultaneous recording of tension and spectra. This was accomplished by drilling holes (2 mm diameter) in the side faces of the cavity. A fibre bundle (6-10 fibres) was mounted in a capillary that was inserted through the cavity, so that the long axis of the bundle was aligned along the magnetic field of the spectrometer. One end of the fibre was secured by surgical silk to a solid-state tension transducer mounted on one side of the cavity. A force was exerted on the other end of the fibre using weights that were attached by a length of surgical silk. Each spectrum was acquired in 4 min, and during this time the fibre did not lengthen appreciably. The derivative of the absorption is plotted as a function of the magnetic field strength, thus the splitting between the three peaks is given by the distance between points where the spectrum crosses the base line, indicated by arrows above the spectra.

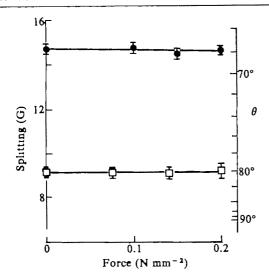


Fig. 2 The splitting between the lines in the electron paramagnetic resonance spectra, indicated by arrows in Fig. 1, is plotted as a function of the stress applied to the fibre. This splitting is a function of  $\theta$ , the mean angle of the angular distribution of the probes, shown on the right. •, Data from fibres labelled with IASL; , data from fibres labelled with MSL. Fibres were labelled with MSL using procedures outlined previously except that the concentration of K<sub>3</sub>Fe(CN)<sub>6</sub>, used to reduce nonspecific labels, was reduced to 25 mM. Each data point represents the mean of at least two separate measurements for each of two to four different fibres. Because of errors in the measurement of the cross-section of the fibre bundles, there is a 20% error in the tension measurements.

the experimental accuracy of the method (±1°) (Fig. 2). The width of the lines in Fig. 1 is related to the spread of the angular distribution. The experimental apparatus used here gave a sharp angular distribution with a full width at half height of  $\sim 12^{\circ}$ . The application of a stress to the fibres resulted in a slight sharpening of the lines, equivalent to further reducing the width of the gaussian distribution by  $\sim 2^{\circ}$ . This effect was found after a small stress was applied, and thus is probably due to better alignment of the fibres than in previous studies<sup>2</sup>. If some of the probes had changed to a new angle, additional intensity would be generated at new positions in the spectrum. No new intensity was seen and a careful comparison of spectra indicated that <10% of the probes changed angles from the gaussian distribution characteristic of rigor muscle.

Although the paramagnetic spectra allow the accurate measurement of the angle of the probe relative to the fibre axis, the relative orientation of the probe and the myosin head is not known with certainty. This uncertainty prompts the question, could the head rotate without probe rotation? Some information on the orientation of the probe relative to the head can be obtained from the measurement of the rotational relaxation time of probes bound to myosin subfragment 1 free in solution. These probes have a long relaxation time of ~200 ns, indicating that their axis lies along some long axis of subfragment 1 (ref. 11). As the myosin head is thought to be an elongated structure with an actin binding site at one end and the attachment to the thick filament at the other, any probe whose axis lies along the long axis of the head should be sensitive to rotations of the head induced by stress. In addition, two paramagnetic probes, linked to the SH<sub>1</sub> through structurally different groups and having axes at least 12° apart, gave the same result. Thus we conclude that the region of myosin surrounding the SH1 does not change orientation when a stress is applied to a rigor fibre. These results agree with those of others 7.8 who also concluded that the myosin head did not rotate on application of stress.

When a step change in length is applied to an isometrically contracting fibre, there is a complex transient in tension of the fibre<sup>3,12</sup>. The first portion of this transient, a sudden change in tension, is interpreted as arising from an elastic element that is in series with the element which generates force. Recent measurements of these transients at different sarcomere lengths have shown that this elasticity resides in the cross-bridge structure and thus may constitute an important part of the contractile apparatus<sup>12</sup>. Some theorists have placed this elastic element in the bond between actin and myosin11, in which case the application of a static stress would change the angle of the crossbridge. Thus our results show that this element cannot reside in the acto-myosin bond and it must lie somewhere between the SH, probe site and the thick filament. As the elastic element is a part of the cross-bridge it may be located in the S2 region which connects the head to the filament backbone, or it may result from some flexibility in the head.

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Present models of the contractile interaction envisage that force is produced by a myosin head attached to actin when the head rotates through an axial gradient of free energy. Whether the contractile element will shift its configuration on application of stress to rigor muscle depends on the shape of the free energy curve for the state in which no nucleotides are bound to myosin. If the configuration of the contractile element is confined in a deep well of free energy in this state then no change is expected. However, if it is confined by a shallower well, such as proposed by some theorists for other states<sup>13</sup>, it should change configuration. Therefore my results require that, unless the contractile element is very stiff in the rigor state, it must also lie between the probe site and the thick filament. This suggests that the actin-myosin bond is stiff and that it is not involved directly in the changes in cross-bridge conformation discussed above.

I conclude that there is a domain of the myosin head whose conformation is not influenced by force on the rigor fibre. As the SH<sub>1</sub> is in this domain, its position provides some estimate of the size of the domain. Although the exact location of the SH<sub>1</sub> is unknown, fluorescent energy transfer has provided some information on its relation to the actin binding site. The distance from SH<sub>1</sub> to Cys 373 of actin has been found to be  $\sim$ 6 nm (ref. 14). The cys 373 is 3.5 nm from the centre of the actin filament whose diameter is estimated to be 7-8 nm (ref. 15), and thus it cannot be very far from the actin-myosin binding site, also shown to be on the outer portion of the actin filament. If these distances are laid out on a three-dimensional model of the structure of the acto-S1 complex16, it appears unlikely that the SH<sub>1</sub> could be directly adjacent to the actin binding site, and thus the domain discussed above probably comprises a significant portion of the myosin head.

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## Synthesis of an unspliced cytoplasmic message by an adenovirus 5 deletion mutant

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Most genes in eukaryotic cells and their viruses contain non-coding intervening sequences (introns)<sup>1-5</sup>. These sequences are excised from each transcript during maturation of mRNA by a process known as RNA splicing. Although very little is known about the mechanism by which splicing occurs or its biological significance, several studies have suggested that the removal of introns is a necessary prerequisite to the accumulation of stable, cytoplasmic mRNA<sup>6-10</sup>. Here we describe a mutant of adenovirus 5 (Ad5) containing a deletion within early region Ela; the deletion prevents splicing of the normal Ela transcripts. The unspliced transcript is found in the cytoplasm of infected cells, showing that for this gene, splicing is not necessary for the biosynthesis of cytoplasmic message.

Region Ela is located at the extreme left-hand end of the adenovirus genome (1.5-4.5 map units). At early times of infection two overlapping mRNAs (a 12S and a 13S species) are produced by differential splicing of a common precursor 11.12. The two messages share 5' and 3' sequences but differ in the extent to which internal sequences are removed by RNA splicing (Fig. 1). The structure of the dl502 genome is also shown in Fig. 1. DNA sequence analysis identified a deletion of 330 base pairs fusing nucleotide 1,009 to nucleotide 1,340. The deletion thus removes the acceptor and one of the donor (D2) splice junctions together with flanking coding sequences.

Because the common acceptor splice junction is absent in dl502, it was anticipated that the primary transcript from this region would fail to undergo splicing. This was found to be the case. Cytoplasmic RNA isolated from wild-type and dl502infected HeLa cells was analysed by the S1 mapping procedure of Berk and Sharp<sup>13</sup> and as the results described in Fig. 2 clearly show, an unspliced Ela message is synthesized and accumulates in the cytoplasm of dl502-infected cells. As the hybridizations are performed with an excess of DNA probe, the intensity of autoradiograph bands is proportional to the abundance of the corresponding mRNA. Comparison of the Ela-generated bands from wild-type and dl502 RNA preparations indicates that the unspliced Ela product from dl502 accumulates in the cytoplasm to 25-50% of the levels of wild-type Ela products. The lower levels are probably due to a defective Ela gene product in dl502. Other mutants with altered Ela products show a similar decrease in the levels of Ela messages, possibly indicating that an Ela product regulates its own synthesis 14,15

We consider it very unlikely that the unspliced message detected could have resulted from nuclear leakage during fractionation of the RNA. The RNA was isolated using isotonic

RNA extraction conditions, where nuclear leakage is reported to be low 16,17. Furthermore, two phenotypic properties of dl 502 that we have studied indicated that a defective Ela gene product is being synthesized and is partially functional. The first property is the host-range phenotype of Dl502. Not unexpectedly, dl502 is defective for growth on HeLa cells. Region Ela encodes a product required for productive growth 18,19; mutants with alterations in this region, including dl502, are grown on 293 cells. These are Ad5-transformed human embryonic kidney cells<sup>20</sup>; they contain and express the left-hand end 11% of the Ad5 genome and consequently will complement mutants which have alterations in this region. Dl502 has a plaquing ratio on HeLa versus 293 cells of  $\sim 10^{-4}$ ; this is  $10^2 - 10^3$ -fold higher than the ratio obtained with another mutant, dl312, which contains a deletion removing almost the entire Ela region (data not shown and ref. 18). We attribute this difference to the synthesis of a partially functional Ela gene product by dl502, supporting our conclusion that a message from this region does accumulate in the cytoplasm of dl502-infected cells.

The second phenotypic property suggesting that a partially functional Ela product is being synthesized by dl502, is that transcripts from other regions of the genome are detected, although at reduced levels. A region Ela gene product is known to regulate the expression of other early regions of the genome, regions E1b, E2, E3 and E4<sup>21,22</sup>. Transcripts from these regions are not detected in dl312-infected cells unless very high MOIs are used<sup>15</sup>. However, as shown in Figs 2 (for region E1b) and 3 (for regions E2, E3 and E4), transcripts from all four of these regions are detected in the cytoplasm of HeLa cells infected with dl502 at relatively low MOIs ( $\sim$ 20 plaque-forming units (PFU) per cell). We again attribute this difference between dl502 and dl312 to the synthesis of a partially functional Ela gene product by dl502.

The major finding of the present study is that an unspliced region Ela transcript accumulates in the cytoplasm of dl502infected cells, showing that splicing and the removal of intron sequences are not necessary prerequisites to the production of stable, cytoplasmic RNA from this particular gene. The characterization of SV40 mutants containing deletions in both early and late genes has led to the conclusion that splicing is required not only for the removal of noncoding intron sequences but also in some unknown way for the stabilization and/or transport of message<sup>6-8</sup>. However, it is now known that several genes, including the gene for adenovirus protein IX<sup>23</sup> and a human interferon<sup>24</sup>, do not contain introns; the synthesis of message from these genes does not therefore require splicing. In addition, two other cases recently have been described where RNA splicing, although normally occurring during the synthesis of message from the genes examined, was found not to be obligatory to the synthesis of message from those genes<sup>25,26</sup>. A viable mutant of SV40, containing a small deletion in the leader region of the late genes, accumulated an unspliced 19S mRNA in the cytoplasm of infected cells25. Interestingly, other mutants containing alterations in different locations and which also prevented splicing of the 19S precursor RNA did not accumulate 19S message<sup>6</sup>, suggesting that the location of the deletion was critical in determining whether splicing was obligatory for

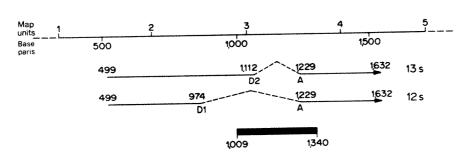
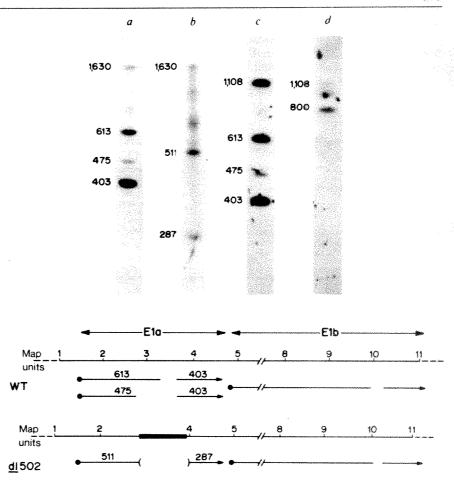


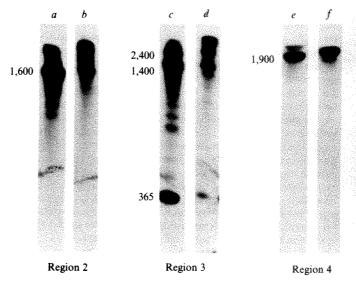
Fig. 1 Structure of the dl502 genome. The position of the deletion in dl502 is shown with respect to the two mRNA species produced from this region early after infection with Ad5<sup>11,12</sup>. The solid line indicates sequences present in the mRNA, caret symbols represent intervening sequences removed by RNA splicing and the arrows mark the position of the 3' end of the RNAs. The nucleotide positions of the splice junctions and 5' and 3' termini are from Perricaudet et al.<sup>27</sup>. D1 and D2 are the terms given to the two donor splice junctions and A is the acceptor junction. The deletion in dl502 as determined by DNA sequence analysis is indicated by the solid bar.

Fig. 2 S<sub>1</sub> analysis of cytoplasmic RNA from wild-type and dl502-infected cells. HeLa cells were infected with purified virions at a MOI of ~20 PFU per cell. Cytosine arabinoside was added 1 h later and the cells were collected 8 h post-infection. Cytoplasmic RNA was isolated from the infected cells by the isotonic extraction procedure described previously RNAs were analysed by the S1 mapping procedure essentially as described by Berk and Sharp<sup>13</sup>. <sup>32</sup>P-labelled DNA fragments (5 µg full genome equivalents per ml; specific activity  $2-4\times10^6$  c.p.m. per µg) were hybridized to total cytoplasmic RNA (5 mg ml<sup>-1</sup>) in 80% formamide, 0.4 M NaCl, 0.04 M PIPES pH 6.4, 0.001 M EDTA at 59 °C for 3 h. The hybridization mixture was then digested with S<sub>1</sub> endonuclease (40 units) in 200  $\mu$ l, 0.03 M NaOAc, 0.25 M NaCl, 0.001 M ZnCl<sub>2</sub>, 5% glycerol at 37 °C for 30 min. The digestion products were ethanol precipitated and electrophoresed on 3.8% polyacrylamide gels containing 8 M urea. a, RNA from wild-type infected cells hybridized to the BglII-D fragment (0-9 map units) isolated from wildtype DNA. Four major S<sub>1</sub>-resistant bands are detected. The band 1,630 nucleotides in length results from hybridization of region E1b mRNA (a region extending over 4.6-11.2 map units<sup>12</sup>) to the probe; the other three bands result from hybridization of E1a mRNA. b, RNA from dl502-infected cells hybridized to the same probe as that used in lane a. A 1,630nucleotide E1b band is seen together with two additional bands, 511 and 387 nucleotides long. These are the expected products if an unspliced RNA from region E1a was synthesized and hybridized to the probe; a deletion loop extending over 2.8-3.8 map units



would be present in the hybrid and be digested with the  $S_1$  endonuclease to give the two fragments seen. c, RNA from wild-type-infected cells hybridized to wild-type HindIII-G fragment (0-7.8 map units). The band resulting from the hybridization of E1b mRNA is smaller with this probe than the one used in lane a (1,108 nucleotides). d, RNA from dl502-infected cells hybridized to the dl502 HindIII-G fragment. As well as the band derived from E1b mRNA, a single additional band of  $\sim$ 800 nucleotides in length was detected which again is the size of the probe sequence that would be protected by an unspliced E1a mRNA. A diagram of region E1 and the mRNAs synthesized from this region by wild-type virus and dl502 is shown. The wild-type RNAs are described in detail in Fig. 1 legend.

Fig. 3 S<sub>1</sub> analysis of cytoplasmic RNA from wild-type and dl502 infected cells using probes specific for early regions 2, 3 and 4. The isolation of RNA and the S<sub>1</sub> mapping procedure are described in Fig. 2 legend. Lanes a, b, hybridization of RNA from wild-type (a) and dl502 (b) infected cells to the HindIII-A fragment (50.1-72.8 map uinits). A major product 1,600 nucleotides long is normally found. Lanes c, d, hybridization of wild-type (c) and dl502 (d) RNA to the HindIII-B fragment (72.8-89.1 map units). Three major products 2,400, 1,400 and 365 nucleotides in length are normally found. lanes e, f, hybridization of wild-type (e) and dl502 (f) RNA to the HindIII-F fragment (89.1-97.1 map units). A 1,900-nucleotide band is the major product. The largest bands detected in all the hybridizations are due to renaturation of the DNA probe; the renaturation is particularly evident in the hybridizations involving dl502 RNA.



message production. It is therefore possible that it is not splicing per se that is the critical factor in producing stable message but the presence or absence of certain important sequences in the precursor.

One possible model to explain all the results would be that a sequence(s) in the precursor RNA is necessary for the synthesis of mature cytoplasmic message (for example by mediating an interaction between the RNA and the nuclear membrane where the transport apparatus and possibly the splicing apparatus resides); if this sequence is missing maturation of the message

from the precursor could not occur. In genes that do not contain introns such a sequence would be located within coding regions. In genes that do contain introns the sequence could be in the intron, the exons or partially in both (perhaps spanning a splice junction). In examples where deletions prevented the accumulation of messages, the model would predict that the necessary sequences had been altered or removed. In examples such as d1502, however, where unspliced messages are found, the prediction would be that the sequence remained and was located within the coding sequences or the remaining portion of the

intron sequences. Implicit in the model is that splicing per se is not necessarily linked to the synthesis of cytoplasmic RNA. Nevertheless, in the normal situation unspliced precursors are confined to the nucleus. This would be the case if the rate of splicing far exceeded that of transport. This model would also be feasible if a particular feature of the secondary structure of the

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precursor RNA was important for message biosynthesis and not simply a primary RNA sequence. The isolation and characterization of additional mutants would help distinguish between these possibilities.

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#### Specific integration of REV proviruses in avian bursal lymphomas

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The most common naturally occurring cancer of chickens associated with retrovirus infection is lymphoid leukosis (LL), a bursa (B) cell lymphoma. The primary causative agents are avian lymphoid leukosis viruses (LLVs), which do not necessarily have an oncogene. Recent evidence of Hayward et al. suggests that LLV infection promotes the expression of a cellular gene, c-myc (homologous to the oncogene of acute leukaemia virus, MC-29) thereby triggering the transformation process. In the majority of the tumours induced by LLV, the provirus is integrated next to the c-myc gene (refs 1, 3, 18, 19). To examine further the specific involvement of c-myc in lymphocytic transformation, we exploited our previous findings that replication-competent or non-defective reticuloendotheliosis virus (nd REV), genetically unrelated to LLV, are also capable of inducing lymphoma in chickens, with similar latent period and pathology to LL5. We have characterized the structure of the nd REV proviruses in the induced tumours and report here that proviral DNA is integrated next to c-myc in over 90% of the tumours analysed. This finding strengthens the hypothesis that the c-myc and its adjacent sequences are important in B-lymphocyte transformation. We also obtained evidence that amplification and structural alteration of the chromosomal region encompassing the REV provirus and cmyc gene have occurred during tumorigenesis in some of the tumours.

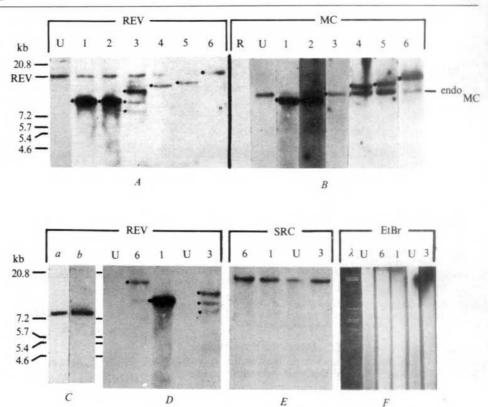
The DNAs extracted from bursal tumours induced by chick syncytial virus (CSV) strain of nd REV were digested with restriction endonucleases, electrophoresed in agarose gels and hybridized to probes specific for the nd REV genome (REVprobe) and the oncogene of MC-29 virus (MC-probe), respectively. The principal enzyme used in this study is EcoRI, which does not cleave the CSV DNA. As shown in Fig. 1C, lanes a and b, the unintegrated linear CSV DNA isolated from the acutely infected cells displays the same migration pattern before and after EcoRI digestion. Similarly, the unintegrated circular DNA can also be shown to lack EcoRI sites (data not shown). When EcoRI was used to cleave the high molecular weight DNA from the tissues (Fig. 1A), in all samples including the uninfected control (lane U), a band of about 18 kilobases (kb) is detected which presumably represents the endogenous REV sequence (endo<sub>REV</sub>). In the tumour sample (lanes 1-6), additional bands (indicated by dots) of differing sizes appear; these are referred to as tumour-specific (TS) bands. The TS band in lane 6, which has a mobility similar to that of endo<sub>REV</sub>, is difficult to discern in this experiment. The endogenous sequence, however, is only weakly hybridizable to the REV-probe (presumably due to the divergence of these two sequences) and it is possible to differentiate the endogenous and exogenous REV sequences by conducting the probe hybridization in more stringent conditions. As illustrated in Fig. 1D, in the more stringent conditions (45 °C) the hybridization to endo<sub>REV</sub> is reduced to the background level (see especially the uninfected control in lane U), whereas the intensity of the TS bands, inducing the 18-kb band of lane 6, are not affected. This analysis confirms the exogenous origin of all the TS bands.

To assess the linkage between REV proviruses and the c-myc gene, the EcoRI cleaved bursal DNAs were hybridized with the MC probe. As shown in Fig. 1B, the TS bands (indicated by dots) detected by the MC probe match the corresponding fragments identified by the REV probe in Fig. 1A. (The band, marked as endo<sub>MC</sub>, corresponds to the endogenous c-myc gene, which is present in both the normal and the tumour tissue<sup>3,6</sup>). Each tumour seems to have one TS band co-hybridizing to both probes. (In tumour no. 3, the TS band which joins the REV provirus and the c-myc gene co-migrates with the c-myc band. The linkage between REV provirus and c-myc gene are confirmed by digestion with other restriction enzymes.) Such cross-hybridizations are not due to homology between REV and MC sequences, as the unintegrated CSV DNA (lane R) is not detected by the MC probe. These results suggest that in each tumour, at least one REV provirus is integrated next to the c-myc gene (on one of the two homologous chromosomes) and strongly implicate the latter gene in lymphomagenesis.

The REV proviruses in 22 of the 25 tumours characterized are found to be linked to c-myc (Fig. 1 and unpublished data). The sizes of the EcoRI linkage fragments vary from 11 to 22 kb. The different sizes of TS bands suggest that integrations of the viral DNAs at several sites near the c-myc gene are conducive to transformation, a conclusion consistent with previous studies of the LLV-induced tumours<sup>1-4,18</sup>. A close examination of the TS band patterns, however, reveals that the size variation of some of these bands may also be caused by deletion or structural alteration of the cellular or viral sequences. If the TS bands result from a simple integrative recombination involving the

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Fig. 1 The structure of REV proviruses and the c-myc gene in lymphoma as analysed using EcoRI digestion. Avian bursal lymphomas were induced by injections of end-point purified, helperindependent chicken cyncytial viruses (CSV; a member of the REV family) intra-abdominally into 1-day-old chicks of line 151, ×71. After a latent period of ~20 weeks, birds which developed lymphomas were killed and the tumour tissues collected<sup>5</sup>. Both the virus stock and the tumour samples were shown serologically and biochemically to be free of avian leukosis viruses. The DNAs were extracted from the tissues, digested with EcoRI, analysed by 0.8% agarose gel electrophoresis and transferred onto nitrocellulose filters, all as previously described15. Filter hybridizations were carried out in 50% formamide and 3× SSC (1×SSC = 0.15 M sodium chloride/0.015 M sodium citrate) at either 35 °C (panels A-C, E) or 45 °C (panel D). The radiolabelled hybridization probes were prepared by nick-trans-16 of the following three pBR322based DNA clones: a, pSNV-SalI 60B (a gift from H. Temin). This clone, which carries the entire genomic sequence (7.7 kb) of spleen necrosis virus (SNV)21 cloned in the SalI site of the pBR322 vector, was used to detect the REV sequence in tumour DNAs (the REV probe). SNV and CSV are genetically closely related and their genomes share ≥80% sequence homology (ref. 14 and M.R.N-D. and H-J.K., unpublished data).



b, pMC-Pst (a gift from D. Sheiness and J. M. Bishop). This clone, which principally carries the oncogene sequence of MC-29 virus<sup>22</sup>, was used to detect the c-myc gene (the MC probe). c, pSRC-pvuIIE (a gift from W. L. Delorbe and H. E. Varmus). This clone, which carries 0.8 kb of the oncogene sequence of Rous sarcoma virus<sup>23</sup>, was used to detect the cellular src gene in chicken chromosome (the src probe). Panels A, B, D, E, EcoRI-digested bursal DNA samples from an uninfected bird (lane U) or from tumorous birds (lanes 1-6). Panel E, the same filter as panel D, after removing the radioactive REV sequences by heating at 90 °C in TE buffer (5 mM Tris pH 6.5, 1 mM EDTA), were hybridized with the src probe as described above. Panel C, the linear, unintegrated CSV isolated from the cytoplasms of chicken embryo fibroblasts infected with the CSV viruses 48 h earlier by the procedures of Shank et al. 15. Lanes a and b represent DNA samples before or after EcoRI (Miles) digestion. All except panel F are autoradiograms of the filters after hybridization with the REV, MC or SRC probes (as indicated at the top of the gel). Panel F shows the ethidium bromide (Etbr)-stained DNA pattern of the gel used to prepare the filter shown in panels D and E. The molecular size markers (in kilobases, kb) shown on the side of the gels correspond to the migration pattern of the EcoRI-digested phage λ DNA (shown in lane λ of panel F), which was included in each gel run as an internal size standard.

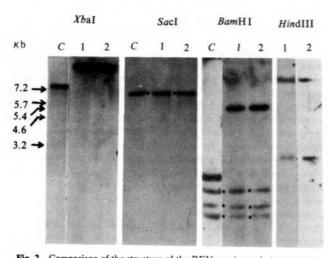


Fig. 2 Comparison of the structure of the REV proviruses in tumours no. 1 and no. 2. Chromosomal DNAs extracted from tumour no. 1 (lane 1), tumour 2 (lane 2) or the cytoplasmic unintegrated CSV DNA (lane C) were digested with restriction endonucleases (Biolabs and Biotech) as indicated at the top of the gels. The samples were analysed in 0.8% agarose gel and the Southern-filter hybridizations with the REV probe were conducted at 45 °C as described for Fig. 1D. Among the enzymes used here, XbaI has no cleavage site, HindIII has one cleavage site and Sac1 and BamHI have multiple cleavage sites in the CSV DNA (ref. 8 and R.A.S. and H-J.K., unpublished data).

REV DNA and the *c-myc* gene without other structural alterations, the *EcoRI-TS* band should have a size equal to the sum of the viral DNA and endo<sub>MC</sub> fragment. We found that this is not the case; especially obvious are samples 1 and 2 where the TS

bands are actually smaller than the c-myc fragment. This result indicates that a deletion or structural alteration (leading to the generation of a new EcoRI cleavage site) must have occurred. Further digestion analyses of the proviruses of samples 1 and 2 using other enzymes, including those which have multiple cleavage sites in viral DNA to generate the internal fragments (for example, SacI and BamHI), are shown in lanes 1 and 2 of Fig. 2. In all cases, identical patterns are displayed by these two proviruses, confirming the identity of the two proviruses. In addition, these proviruses seem to carry the major BamHI and SacI internal fragments (indicated by dots), comigrating with those generated from the cytoplasmic unintegrated viral DNA (Fig. 2, lane C). This indicates that the majority of viral sequences are present in these proviruses. Thus, the structural alterations which gave rise to the 'shortened' 11-kb TS band probably involve the surrounding cellular sequence.

The other interesting feature of the results shown in Fig. 1D is the unusual intensity of the hybridization signal of some of the TS bands, especially the 11-kb band in sample 1. The total amount of the chromosomal DNA loaded on each lane, as judged by the ethidium bromide stains, is comparable (Fig. 1F). Furthermore, when the same samples were hybridized with a cloned DNA carrying a portion of v-src (the transforming gene of Rous sarcoma virus), the intensity of the cellular src genes seem to be similar in all lanes. This confirms that the amount of chromosomal DNA of lane 1 is not appreciably different from that of other lanes. The high intensity of the 11-kb TS band in sample 1 is revealed by both REV and MC probes (Fig. 1A, B). This observation, together with the larger size of this band relative to the unintegrated viral DNA, suggests that amplification of the REV provirus and its surrounding cellular

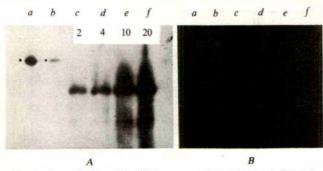


Fig. 3 Quantification of the CSV sequences in tumour no. 1. Panel A, 30 µg each of the EcoRI-digested chromosomal DNA from tumours no. 1 (lane a) and no. 8 (lane b) were analysed on a 0.8% agarose gel, then hybridized with the REV probe. The DNA concentration was determined by diphenylamine assay<sup>17</sup>. The cloned pSNV-Sal-60B DNA (lanes c-f) was used as a quantitation standard. The DNA insert which carries the 7.7-kb viral sequence was purified from the pBR322 vector by SalI cleavage and isolated from a preparative agarose gel. Lanes c, d, e and f contain 0.23, 0.46, 1.15 and 2.30 ng each of the purified SNV DNA insert. Based on a diploid genome size of 2×109 bases for chicken, these amounts of SNV DNA would give a 2, 4, 10 and 20 copies per cell equivalent of CSV sequences in 30 µg of chicken DNA. The weak, lower band in lanes c-f represents the contaminating pBR322 vector DNA. Panel B, the ethidium bromide-stained gel of panel A.

sequences have occurred. To obtain a further estimate of the amount of CSV sequences present in tumour no. 1, a titration experiment using the pSNV DNA (the plasmid clone used to make REV probe; see Fig. 1 legend) as quantitation standards was carried out (Fig. 3A). Thirty microgrammes each of tumours no. 1 (lane a) and no. 8 (lane b) DNA, digested with EcoRI, were loaded on an agorose gel together with varying amounts of the purified 7.7-kb insert of the pSNV DNA (lanes c-f). The quantities of the cloned DNA in lanes c-f were such that they represent, respectively, 2, 4, 10 and 20 copies per cell equivalent of CSV sequence in 30 µg of chicken DNA. We included tumour no. 8 DNA as a control, because it has an EcoRI-TS band similar in size to that of tumour no. 1, but has a usual, non-amplified intensity. (The ethidium bromide-stained gel is shown in Fig. 3B.) When the TS bands in lanes a and b were traced densitometrically and their intensity compared with that of the standards in lanes c-f, it was found that while tumour no. 8 acquired only one copy of the CSV provirus, tumour no. 1 appears to carry approximately eight copies of the proviral sequences.

The observation that the REV provirus and its adjacent cellular sequences may have undergone structural alteration in some of the tumours is intriguing in the light of the recent findings that retroviral DNA has a transposable-element-like structure<sup>8-12</sup> which behaves like prokaryotic transposable-elements in showing, for example, deletion formation and insertional mutagenesis 13,20. It is not clear how such structural alterations contribute to the transformation process, if indeed they do. Detailed structural analysis of the molecularly cloned TS bands should provide insight into the molecular mechanism of the recombination and their possible functions in transformation. Finally, the endogenous REV sequences detected in Fig. 1A merits discussion; such an EcoRI fragment has not previously been reported, although earlier liquid hybridization studies indicated a low level of homology (10-20%) between REV and the normal chicken chromosome<sup>14</sup>. We do not know whether this endogenous REV sequence is specific for the inbred line used here or is ubiquitous in chickens. We are now conducting restriction enzyme digestion analysis of the endo<sub>REV</sub> to determine the structural relationship between the endogenous and exogenous viral genomes.

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#### An isolated pseudogene related to the 5S RNA genes in Neurospora crassa

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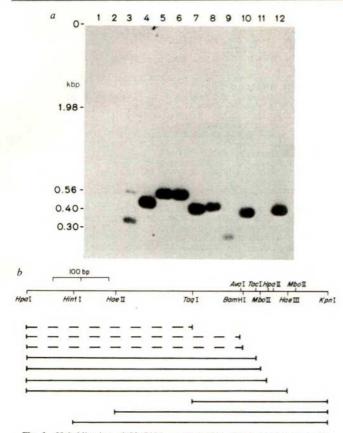
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DNA segments which do not code for functional products but are similar in sequence to DNA of known function are called 'pseudogenes'. Most pseudogenes discovered so far are related to members of gene families: 5S RNA (5S) genes in Xenopus 1-3 globin genes in various mammals4-6, actin genes in Dictyostelium7 and small nuclear RNA genes in man8. Presumably, in the absence of strong selective forces, redundant genes drift apart and occasionally become nonfunctional. Of course, mechanisms which maintain homogeneity in multigene families should tend to prevent the occurrence of pseudogenes. There is ample evidence for 'horizontal' or 'concerted' evolution in tandemly repeated gene families<sup>9-11</sup>. The 5S pseudogenes of Xenopus laevis, of which there is a copy in every tandemly repeated unit, may be a rare example of a defective gene not eliminated from the family by unequal crossing-over or gene conversion events. We have recently found evidence for limited concerted evolution in a dispersed gene family, the 5S genes of Neurospora crassa 12: both identical and highly divergent 5S coding regions were discovered. We report here that we have found a pseudogene related to the Neurospora 5S genes. This pseudogene, N5SP1, contains a segment of DNA almost identical to the first 50 nucleotides of the major species of Neurospora 5S RNA. The sequences flanking this region are totally different from sequences found within or adjacent to full-length 5S genes. Hybridization experiments suggest that N5SP1 is not transcribed. The existence of this pseudogene is consistent with our transposition model for horizontal evolution in dispersed gene families12.

N5SP1 is contained on a plasmid designated pMF4. It was identified in a collection of Neurospora DNA fragments cloned into pBR322 (ref. 13) by colony hybridization 14 using labelled 5S RNA as probe. The first indication that pMF4 does not contain a complete 5S gene came from Southern 15 hybridization

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Hybridization of 5S RNA to restriction endonuclease digests of pMF4 DNA. a, Plasmid DNA (2 µg) was digested with KpnI and HpaI, together with Aval (lane 1). BamHI (lane 2), HaeII (lane 3), HaeIII (lane 4), HhaI (lane 5), HincII (lane 6), HpaII (lane 7), HinfI (lane 8), TaqI (lane 9), Tacl (lane 10), Sau3A (lane 11), or MboII (lane 12). The resulting DNA fragments were resolved on a 1.5% agarose gel in Tris-EDTA-acetate-NaCl buffer<sup>23</sup> and transferred to a nitrocellulose filter according to the method of Southern15. The filter was then hybridized with 32P-labelled 5S RNA $^{12}$  at 65 °C overnight in 5×SSC, 0.1% SDS. It was then washed extensively with 5×SSC, blot-dried and autoradiographed. The positions of size standards (in kilobase pairs, kbp) and of the origin (O) are indicated. HhaI (lane 5) and HincII (lane 6) did not cleave the KnpI-HpaI DNA fragment and the HaeII digestion (lane 3) did not go to completion. The DNA fragments which hybridized to 5S RNA in lanes 3 and 9 were too small to transfer effectively to nitrocellulose and therefore produced weak bands. b, Restriction map of KpnI-HpaI segment of pMF4 (data not shown) and interpretation of hybridization results. Solid horizontal lines indicate DNA fragments which hybridized to 5S RNA and broken lines indicate fragments which failed to hybridize. Fragments smaller than the TaqI-KpnI fragment did not transfer efficiently, and thus cannot be scored. Note that the Hpal-Tacl fragment hybridized well with 5S RNA, whereas the 19-nucleotide shorter HpaI-BamHI (or Sau3A) fragments did not hybridize at all. This is presumably because the BamHI cut is in the middle of the largest uninterrupted stretch of homology to 5S RNA.

experiments. Restriction digests of pMF4 DNA were fractionated by agarose gel electrophoresis, transferred to a nitrocellulose filter and hybridized with 32P-labelled 5S RNA. The results of this experiment showed that 5S RNA hybridizes to pMF4 DNA within a 550-base pair (bp) KpnI-HpaI fragment located near the middle of the 5,400-bp Neurospora DNA insert (data not shown). Surprisingly, digestion of pMF4 DNA with BamHI, which cleaves the KpnI-HpaI fragment, eliminates all subsequent hybridization with 5S RNA. Functional Neurospora 5S genes contain a BamHI site at nucleotide 32 in the coding region, but DNA cleaved at this site still hybridizes to 5S RNA 12. These findings suggest that a Bam HI site in pMF4 is in a region of incomplete 5S homology. The DNA segment homologous to 5S RNA was mapped by Southern hybridizations of pMF4 DNA digested with KpnI and HpaI together with other restriction endonucleases. The results (Fig. 1a) are consistent with the idea that the region of 5S homology is near the BamHI site (Fig. 1b). This region was sequenced as indicated in Fig. 2a and compared with sequences of two Neurospora 5S genes (Fig. 2b). As expected, the BamHI site is in a region homologous to 5S RNA. A ~ 50-nucleotide segment

corresponds to the first  $\sim 40\%$  of 5S RNA. With the exception of a three-nucleotide insertion, a single nucleotide insertion and two single nucleotide substitutions, this region matches the major 5S RNA sequence found in *Neurospora*. Outside this segment, there is no significant homology with DNA in or around the *Neurospora* 5S genes. *Neurospora* mitochondrial DNA does not hybridize to pMF4 (data not shown), ruling out the possibility that the cloned DNA encodes a mitochondrial RNA similar to cytoplasmic 5S RNA.

The Southern hybridization experiments and DNA sequence analysis suggests that pMF4 does not encode full-length 5S RNA. S<sub>1</sub> nuclease mapping <sup>16</sup> was performed to verify this (Fig. 3). pFM4 DNA 3'- or 5'-labelled at the Bam HI site in the region of 5S homology was hybridized with excess 5S RNA in R-looping <sup>17</sup> conditions. The single-stranded regions were digested with S<sub>1</sub> nuclease and the resulting hybrids denatured, fractionated on a denaturing polyacrylamide gel and autoradiographed. DNA fragments protected by RNA were detected on one side of the Bam HI site (corresponding to the 5' portion of 5S RNA) but not on the other, confirming that pMF4 does not encode a 5S RNA. For the authentic 5S clones pKD51 and pKD52, fragments corresponding to both the 5' and 3' portions were evident (Fig. 3).

To determine whether the presumptive pseudogene encodes RNA of a different size from 5S RNA, total *Neurospora* RNA fractioned on native (2.1% polyacrylamide, 0.5% agarose) or denaturing (7 M urea, 10% polyacrylamide) gels was transferred to diazobenzyloxymethyl paper 18,19, and probed with labelled pMF4 DNA. In each case, the only RNA species detected corresponded to 5S RNA, so it seems likely that the 5S-like DNA segment in pMF4 is a 5S pseudogene, which we designate N5SP1.

The sharp break in the sequence homology between N5SP1 and 5S RNA presumably reflects a DNA rearrangement which replaced the 3' portion of the ancestral 5S gene with foreign DNA. This substitution separated the 5' portion of the 5S gene from the central region which, at least in X. laevis 5S RNA genes<sup>20-22</sup>, is necessary for transcription initiation. A gene inactivated by such a rearrangement might be expected to accumulate subsequent mutations. The small insertions and point changes in N5SP1, relative to active 5S genes, presumably are examples of this. Other pseudogenes which have been discovered<sup>1-8</sup> also contain similar mutational changes.

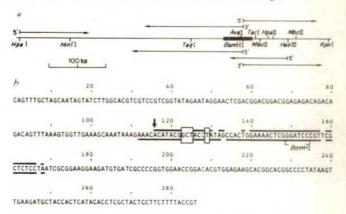


Fig. 2 DNA sequence of Neurospora 5S pseudogene, N5SP1. a, Restriction map with horizontal arrows illustrating sequencing runs on the two strands of DNA (above and below map). The lengths of the arrows indicate the extent to which the sequence could be read with confidence. DNA fragments were labelled at 5' termini by phosphorylation using  $[\gamma^{-32}P]$  ATP and T4 polynucleotide kinase<sup>21</sup> or at 3' termini by 'filling in' using  $[\alpha^{-32}P]$ dGTP and Escherichia coli DNA polymerase  $I^{24,25}$ . They were then sequenced according to the methods of Maxam and Gilbert<sup>26</sup>. Reaction products were resolved on 0.35-mm thick 20% or 8% polyacrylamide gels containing 7 M urea<sup>27</sup>. The heavy line indicates the region of homology to 5S RNA. b, DNA sequence of one strand in the vicinity of the BamH1 site in N5SP1. The horizontal lines above and below the sequence show the extent of homology with Neurospora β and α 5S genes on pKD51 and pKD52, respectively<sup>12</sup>. Boxes enclose two small insertions relative to the 5S genes. The arrow shows the presumed position of transcription initiation in the 5S genes.

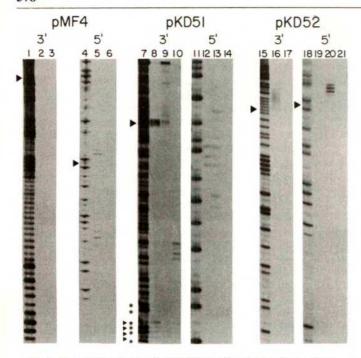


Fig. 3 S<sub>1</sub> nuclease mapping of 5S RNA homology to pseudogene clone pMF4 and authentic 5S clones pKD51 and pKD52. The BamHI ends of DNA fragments Kpnl-BamHI (lanes 1-3) and Pstl-BamHI (lanes 7-10 and 15-17) were labelled at their 3' ends (see Fig. 2). The BamHI ends of the fragments TaqI-BamHI were labelled at their 5' ends (lanes 4-6, 11-14 and 18-21). The labelled DNA was then used for the G>A DNA sequencing reaction 26 (lanes 1, 4, 11, 15, 18) or the A>C reaction (lane 7), or hybridized in R-looping conditions (10 µl containing 0.4 M NaCl, 0.04 M PIPES pH 6.4, 1 mM EDTA and deionized 80% formamide; 55 °C for the 5' fragments and 65 °C for the 3' fragments), with (lanes 2, 5, 8, 9, 12, 13, 16, 19, 20) or without (lanes 3, 6, 10, 14, 17, 21) a  $\sim$ 100-fold excess (0.1  $\mu$ g) of 5S RNA. After 16 h, the samples were chilled, diluted 10-fold in 0.25 M NaCl, 0.03 M sodium acetate pH 4.6 and 100 µg ml-1 denaturated salmon sperm DNA, and treated with  $S_1$  nuclease (Boehringer) at  $1~U~\mu l^{-1}$  (lanes 2, 3, 5, 6, 8, 10, 13, 14, 17, 20, 21) or  $10~U~\mu l^{-1}$  (lanes 9, 12, 16, 19) at  $22~^{\circ}$ C for 20~min. Finally, the nucleic acids were ethanol-precipitated, rinsed with 70% ethanol, dried and resuspended in 5-10 µl deionized 99% formamide containing xylene cyanol and bromophenol blue and fractionated on 8% or 20% polyacrylamide sequencing gels<sup>27</sup> next to the same DNA which had undergone partial cleavage in a DNA sequencing reaction. Large arrowheads indicate the expected migration of the fragments from the BamHI site to 3' or 5' ends of the 5S coding regions of pKD51 and pKD52. Small arrowheads mark the positions of faint bands in lane 8 where bulk 5S RNA fails to protect completely pKD51. This is near a region (\*) of recognized12 non-identity between pKD51 and pKD52.

If a defective 5S gene such as N5SP1 were part of a tandemly repeated multigene family, it could be eliminated by unequal crossing-over. Alternatively, it could be passively amplified as apparently occurred in the case of the Xenopus laevis 5S pseudogene 1,2. In dispersed gene families, defective members cannot be lost by unequal crossing-over. However, there is evidence for limited concerted evolution among the dispersed Neurospora 5S genes<sup>12</sup>. We pointed out that gene conversion or gene transposition, together with normal recombination processes, should have a homogenizing effect in a family of dispersed, repeated genes12. A feature of our model for concerted evolution by conservative transposition is that genes which fail to jump are immune to the homogenizing mechanism. The Neurospora 5S pseudogene may be an example of a defective gene which no longer participates in concerted evolution. Presumably it will continue to drift until it either becomes functional in some new way, or is deleted.

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#### Identification of the Escherichia coli recB and recC gene products

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Functional recB and recC genes are required for normal conjugative genetic recombination in Escherichia coli<sup>1-3</sup> mutations in either of these genes lead to deficiency in an ATP-dependent nuclease known as the RecBC DNase or exonuclease V (refs 4-6). Widely different molecular weights have been reported for the putative subunits of this enzyme<sup>7,8</sup> and genetic and biochemical experiments have left some doubt about whether or not recB and recC are different genes. We have now cloned the recB and recC genes separately into the multiple copy number plasmid pAT153 and identified the gene products by transposon inactivation and gel electrophoresis. The products of the recB and recC genes are proteins of molecular weights (MW) 135,000 and 125,000, respectively.

Mutations in either the recB or recC genes lead to apparently identical deficiency in genetic recombination, increased radiation sensitivity and decreased post-irradiation DNA degradation1,2. The recB and recC mutations cannot be distinguished phenotypically but have been assigned to different genes on the basis of genetic complementation tests and co-transduction frequencies with the neighbouring argA and thyA genes3. However, complex complementation behaviour has been observed among several recombination deficient mutations in this region (Hoekstra et al. cited in ref. 9) and the possibility that the observed complementation between recB and recC mutations may be intragenic cannot be discounted. In vitro complementation studies have also been difficult to interpret. The RecBC enzyme has been partially purified and dissociated by high salt concentrations into two subunits of MW 60,000 and 170,000, as determined by sedimentation in glycerol gradients<sup>7</sup>. It was found that the 170,000-MW protein would complement extracts from both recB and recC mutant strains whereas the 60,000-MW protein complemented neither7. These results suggest that the 170,000-MW protein is either a single polypeptide encoded by one gene of which recB and recC are mutations, or that it is made up of two polypeptides encoded by recB and recC which are not dissociated by high salt concentrations. However, in other work8, the enzyme has been purified to about 90% homogeneity and preparations were found, by SDS-polyacrylamide gel electrophoresis, to consist mainly of two protein subunits of 128,000 and 140,000 MW.

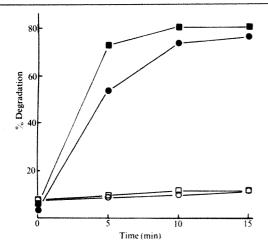


Fig. 1 ATP-dependent DNase activity in cell extracts. ○, AB2470 (recB); ♠, AB2470 (recB) harbouring pPE230 (recB<sup>+</sup>); □, AB3058 (recC); ■, AB3058 (recC) harbouring pPE220 (recC<sup>+</sup>). Cell lysates were assayed<sup>24</sup> for ability to degrade <sup>3</sup>H-labelled phage λ DNA to trichloroacetic acid-soluble material in the presence of ATP. Less than 10% degradation occurred in extracts of all four strains in the absence of ATP. Results with the strain AB1157 (rec<sup>+</sup>) were similar to those with AB2470 (pPE230) (data not shown).

In work to be published elsewhere, we first cloned a BamHI fragment of chromosomal DNA which included recC<sup>+</sup> into a phage λ vector to produce a recombinant phage λdrecC which was then used to derive the phage λdrecBC by secondary site integration at recC followed by aberrant excision (I.D.H., K. E. Atkinson and P.T.E., in preparation). We then subcloned the recB and recC genes from this phage DNA separately into the BamHI site of the plasmid vector pAT153 (Apr, Tcr) and transformed AB2470 (recB21) and AB3058 (recC22). Colonies resistant to both ampicillin and mitomycin C were selected and screened for those that were sensitive to tetracycline and resistant to UV light. The plasmids pPE230 (recB) and pPE220 (recC), obtained in this way, when transformed into AB2470 and AB3058, restored wild-type levels of RecBC

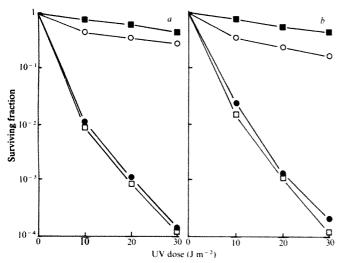


Fig. 2 UV-survival curves showing complementation of the recB mutation by pPE230 (recB<sup>+</sup>) and the recC mutation by pPE220 (recC<sup>+</sup>). a, ○, AB3058 (recC22) harbouring pPE220 (recB<sup>+</sup>); □, AB3058 (recC22) harbouring pPE230 (recB<sup>+</sup>); □, AB3058 (recC22) harbouring pAT153; ■, AB1157 (rec<sup>+</sup>) harbouring pAT153. b, ○, AB2470 (recB21) harbouring pPE230 (recC<sup>+</sup>); □, AB2470 (recB21) harbouring pPE220 (recC<sup>+</sup>); □, AB2470 (recB21) harbouring pAT153; ■, AB1157 (rec<sup>+</sup>) harbouring pAT153. The bacteria were grown to mid-exponential phase, collected, serially diluted in buffer, spread on nutrient agar plates, irradiated with a low-pressure mercury germicidal lamp and incubated overnight at 30 °C.

enzyme activity (Fig. 1) and UV resistance (Fig. 2). Restriction maps of these plasmids are shown in Fig. 3.

To identify the products of the recB and recC genes, we inserted the  $\gamma\delta$  (ref. 10) sequence of the sex factor F from Flac into the genes and examined extracts of 'maxicells' for altered proteins 11,12. The strain NH4104 (F'lac, Str\*) harbouring either pPE220 or pPE230 was mated with AB3058 (recC22) or AB2470 (recB21), respectively, and ampicillin-resistant, streptomycin-resistant exconjugants were selected. This selection is for plasmids (which carry Apr) transferred on conjugation because they have acquired  $\gamma\delta$  insertions from the F DNA. Colonies were then picked and screened for those which were UV-sensitive because the inserted transposable element had inactivated the recB or recC gene on the plasmid, which was therefore no longer able to complement the chromosomal mutation. Plasmid DNA was isolated<sup>13</sup> to confirm that the νδ sequence was inserted into the original plasmid, as manifested by an increase in size.

The proteins encoded by pPE220 and pPE230 were examined by subjecting labelled 'maxicell' extracts to SDS-polyacrylamide gel electrophoresis and fluorography. The results (Fig. 4) show that the plasmid pPE220 (recC) encodes a polypeptide of MW 125,000 (track 6) that is missing in plasmids in which the recC gene is inactivated by  $\gamma\delta$  (tracks 8–10). This polypeptide is present in a plasmid which carries a  $\gamma\delta$  insert in a gene other than recC (track 7). Similarly, the plasmid pPE230

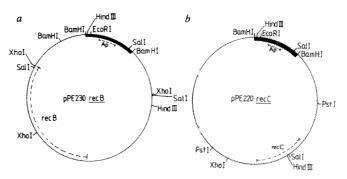
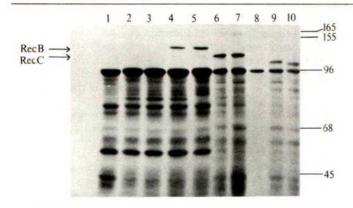


Fig. 3 Restriction maps of the recombinant plasmids. a, pPE230 (recB); b, pPE220 (recC). Vector DNA is drawn with a bold line. The inserts are  $\sim 17.5$  and 21.5 kilobases in pPE230 and pPE220, respectively. Restriction analysis of the plasmids and of their derivatives in which  $\gamma\delta$  is inserted into the recB and recC genes shows that these genes are located within the regions indicated.

(recB) encodes a polypeptide of MW 135,000 (track 5) that is absent in plasmids in which  $\gamma\delta$  is inserted into the recB gene (tracks 1-3), but present in a plasmid in which it is inserted at a site other than the recB gene (track 4). Insertion of  $\gamma\delta$  into a gene results in termination of transcription in five out of the six possible reading frames <sup>12,14</sup>, giving rise to truncated peptides. Truncated peptides can be seen in tracks 9 (115,000 MW) and 10 (108,000 MW). Proteins of MWs 125,000 and 135,000 are also synthesized in heavily UV-irradiated host cells infected with λdrecBC while a protein of MW 125,000 but not one of 135,000 is synthesized in similar experiments with the phage λdrecC which lacks recB (I.D.H., K. E. Atkinson and P.T.E., in preparation).

We conclude that the  $recB^+$  gene product is a protein of MW 135,000, the  $recC^+$  gene product is a protein of 125,000, and that recB and recC are different genes. Further evidence that these are different genes is provided by restriction analysis (Fig. 3), which fails to reveal any similarities between the restriction patterns of the inserts, and the complementation analysis (Fig. 2), which shows that there is a very sharp distinction between the abilities of pPE230 (recB) and pPE220 (recC) to complement recB and recC mutations. Our results do not support the conclusion that the recB and recC genes are both required to produce one of the subunits of the RecBC enzyme, but suggest that the two peptides of MWs 140,000 and 128,000 found in



<sup>35</sup>S-methionine labelled proteins produced in maxicells by the plasmids pPE230 (recB+) and pPE220 (recC+) and their derivatives in which  $\gamma\delta$  is inserted in the recB or recC gene  $(\gamma\delta 1-3)$ or in a gene other than recB or  $recC(\gamma\delta 0)$ , Tracks: 1, pPE230- $\gamma\delta 3$ ; 2, pPE230-γδ2; 3, pPE230-γδ1; 4, pPE230-γδ0 (recB<sup>+</sup>); 5, pPE230 (recB<sup>+</sup>); 6, pPE220 (recC<sup>+</sup>); 7, pPE220-γδ0 (recC<sup>+</sup>); 8, pPE220-γδ1; 9, pPE220-γδ2; 10, pPE220-γδ3. Proteins were separated by electrophoresis on an 8% polyacrylamide gel and visualized by fluorography. Molecular weights, determined by reference to unlabelled protein standards followed by staining, are indicated. The standards were RNA polymerase  $\beta'$  (165,000), RNA polymerase  $\beta$  (155,000), phosphorylase b (96,000), bovine serum albumin (68,000) and ovalbumin (45,000).

preparations of the RecBC enzyme that were 90% pure were the recB and recC products, respectively.

Our cloning of the recB and recC genes individually into a multicopy plasmid may lead to an easier method of isolating the individual subunits of the RecBC enzyme and should permit investigation of their partial properties. Our results do not rule out the possibility that active RecBC DNase includes one or more subunit(s) encoded by a gene or genes other than recB and recC. However, purification of the individual RecB and RecC subunits will permit studies of the reconstituted enzyme to help answer this question. Recent results show that at physiological ATP concentrations and in the presence of Ca<sup>2+</sup> the RecBC DNase uses energy derived from ATP hydrolysis to travel through duplex DNA, unwinding the DNA ahead of itself and rewinding it behind 15-17. In this regard, the RecBC DNase resembles other enzymes such as the helicases, gyrase and RecA protein which use the energy derived from ATP hydrolysis to modify the physical structure of DNA. Cloning of the recA gene 18-20 and the resultant ease with which the RecA protein could be isolated paved the way for the biochemical experiments which have greatly helped to explain the role of the RecA protein in genetic recombination and DNA repair<sup>21-23</sup>. The cloned recB and recC genes may also facilitate the isolation of the RecBC DNase and stimulate further experiments to determine its role in the cell.

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#### Sexual activity reduces lifespan of male fruitflies

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Many theories on the evolution of life histories have assumed a physiological cost of reproduction in terms of reduced lifespan 1-3. A cost of increased reproduction in terms of reduced longevity has been established experimentally for females, both as an additive genetic<sup>4,5</sup> and as a purely phenotypic<sup>6,7</sup> effect. Such a physiological cost of reproduction has not been demonstrated for males. The cost of sexual activity has been assumed to be relatively small in those species where the only paternal contribution to an offspring is the gamete8.9. Here we show that increasing sexual activity reduces longevity in the male fruitfly (Drosophila melanogaster) and hence that there is a significant physiological cost of male sexual activity in a species where the father contributes only gametes to his progeny.

The flies used were an outbred stock collected in Dahomey in 1970. Sexual activity was manipulated by supplying individual males with receptive virgin females at a rate of one or eight virgins per day. The longevity of these males was recorded and compared with that of two control types. The first control consisted of two sets of individual males kept with newly inseminated females equal in number to the virgin females supplied to the experimental males. Newly inseminated females will not usually re-mate for at least 2 days 10,11 thus they served as a control for any effect of competition with the male for food or space. The second control was a set of individual males kept with no females. There were 25 males in each of the experimental and control groups, and the groups were treated identically in respect of number of anaesthetizations (using CO2) and provision of fresh food medium.

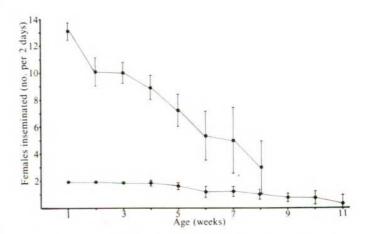
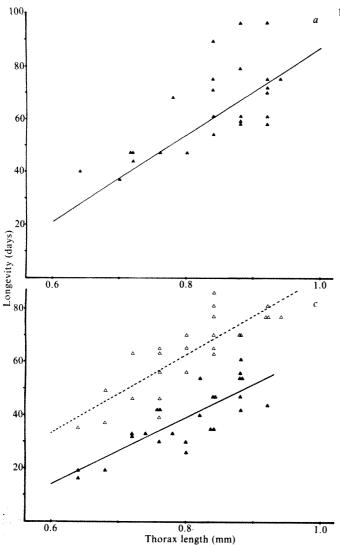


Fig. 1 The relationship between insemination rate (number of females inseminated per 2 days) and age (weeks) for males kept with one virgin female ( ) or eight virgin females ( ) per day. Error bars are 95% confidence limits.



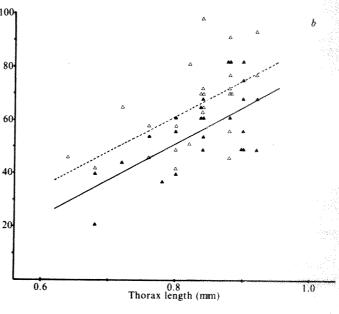


Fig. 2 The relationship between longevity and size for a, males kept with no females; b, males kept with one virgin (A one inseminated (△- $--\triangle$ ) female per day; c, males kept with eight virgin (▲- $-\triangle$ ) or eight inseminated  $(\triangle - - - - \triangle)$  females per day. The lines show the regression of longevity on thorax length, which is statistically significant (P < 0.01) in each case. Analysis of covariance showed that none of the control groups (no females, or one or eight inseminated females per day) differed significantly from one another in longewity. Males kept with one virgin female per day had reduced longevity compared with males kept with no females (P < 0.05) or one inseminated (P < 0.01)female per day; those kept with eight virgin females per day had reduced longevity compared with males kept with no females (P < 0.001), eight inseminated females (P < 0.001) or one virgin female (P < 0.001) per day.

On two days per week throughout the life of each experimental male, the females that had been supplied as virgins to that male were kept and examined for fertile eggs. This gave an estimate of the insemination rate of the two groups of males (Fig. 1). In both groups the insemination rate declined with the age of the male, and the rate was higher for males supplied with eight virgins per day than for those supplied with only one virgin per day. There were no significant differences in insemination rate between the individual males within each experimental group.

In the absence of any sexual activity, the longevity of male fruitflies is associated with their size (Fig. 2a). Size was therefore taken into account when examining the effect of sexual activity on longevity (Fig. 2b, c). Analysis of covariance showed that: (1) there were no significant differences in longevity between the control groups (median longevity 65 days); (2) the males supplied with one virgin female per day had significantly reduced longevity (median 56 days) compared with males in any control group; (3) males kept with eight virgin females per day had significantly reduced longevity (median 40 days) compared with males kept with one virgin female per day, and control males. These results show that male sexual activity reduces longevity, and that this effect is more marked for a higher level of sexual activity.

Physiological costs of particular activities have generally been discussed in terms of the diversion of nutrients into these activities at the expense of others<sup>12</sup>. In our experiment, energetic costs of sexual activity would have included the production of sperm and seminal fluid and the muscular action associated with mating itself. In addition to inseminating females, the

experimental males probably also performed higher levels of courtship than control males. The control males kept without females performed no courtship. When inseminated females are courted they extrude the ovipositor, which terminates the male courtship<sup>13</sup>. In nature, nutritional effects may be increased by food shortage, and there may be costs of sexual activity in addition to those detected here. Searching for mates<sup>14</sup> and fighting with other males<sup>15</sup> may be costly physiologically and these activities, together with courtship and mating, may make males more vulnerable to predation<sup>16</sup>.

Sexual activity could affect longevity in two ways. First, it may have an effect on the probability of death occurring in the next short period of time. Cessation of sexual activity at any age would then leave a fly with a life expectancy comparable with that of controls of the same age. High temperatures can have such an effect on longevity in *Drosophila subobscura* 17. Second, sexual activity may have some cumulative, possibly irreversible, effect. Williams 18 has suggested that senescence may be caused by the deleterious pleiotropic effects later in life of genes which have beneficial effects early in life. A deleterious long-term effect of sexual activity earlier in life could produce such a pleiotropic effect. Phenotypic correlations of the kind found in these experiments need not necessarily mean that a genetically caused change in the level of male sexual activity would alter longevity. A negative additive genetic correlation would be needed to demonstrate this and should be the subject of further experiments.

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#### Pulmonary vein as an ectopic focus in digitalis-induced arrhythmia

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The tunica media of the pulmonary vein (PV) in many mammalian species, including man, is made up of cardiac muscle<sup>1</sup> Brunton and Fayrer first reported that independent pulsation of the PV occurred in cats and rabbits even after activity of the heart had ceased4. Electrophysiological studies have also shown spontaneous electrical activity in isolated PVs of the guinea pig and a pacemaking region has been located at the distal end of the cardiac portion of the vein adjoining the smooth muscle. The intrinsic frequency of the PV was low and activity at the PV was normally coupled to the sinus rhythm<sup>5</sup>. Thus, the PV behaves as a subsidiary pacemaker. In the presence of digitalis-type agents, subsidiary pacemakers such as Purkinje fibres develop oscillatory afterpotentials (OAPs) which may be large enough to reach threshold<sup>6,7</sup>, and it has been suggested that OAPs at Purkinje fibres leading to spontaneous action potentials may provide the underlying mechanism for ventricular arrhythmia during digitalis intoxication<sup>6</sup>. In contrast, atrial and ventricular muscles are not very sensitive to the arrhythmogenic action of digitalis8. I have therefore now investigated whether the PV can develop OAPs and act as an ectopic focus with digitalis intoxication. By recording with intracellular microelectrodes simultaneously at the PV and right atrium, I demonstrate that OAPs and repetitive activity develop at the PV in the presence of ouabain. Propagation of these triggered action potentials at the PV into the atrium results in atrial extrasystoles.

The recording arrangements in these experiments were similar to those reported previously<sup>5</sup>. Using male guinea pigs (300-400 g), intracellular recordings were made with glass micropipettes filled with 3 M KCl. All recordings were made at the distal end of the cardiac PV and the dorsal surface of the intact right atrium close to the vena cava. The experiments were carried out at 32-34 °C, the preparations being superfused continuously with normal or ouabain-containing physiological solutions at a rate of 4 ml min<sup>-1</sup>.

In spontaneously active preparations, the PV was driven by impulses originating from the SA node<sup>5</sup>. In the presence of ouabain (0.5–1.0  $\mu M$ ), the sinus rate decreased and became less regular, and in all seven preparations studied, after 30-40 min OAPs developed at the PV but not in atrial muscle cells (Fig. 1). Diastolic potentials between action potentials remained flat at the atrial recording site.

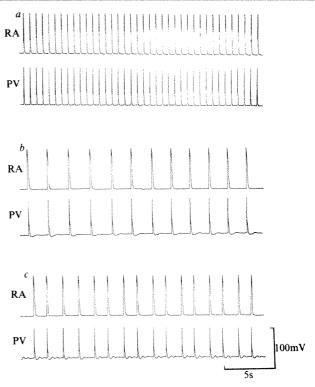


Fig. 1 Effect of ouabain  $(0.5 \mu M)$  on the electrical activity of the pulmonary vein (PV) and the right atrium (RA). As demonstrated previously, action potentials at the PV are closely coupled to those at the RA5. After exposure to ouabain for 40 min, OAPs developed at the PV (b) and became very prominent at 50 min (c). No membrane oscillation was observed at the RA. The spontaneous frequency of the preparation decreased in the presence of ouabain.

When higher ouabain concentrations were used ( $\geq 2 \mu M$ ), high frequency repetitive activity appeared in six out of six preparations. Figure 2 demonstrates the onset of repetitive activity in a preparation after exposure to ouabain (2.5 µM) for 75 min. Initially, two short bursts of extrasystoles were observed (Fig. 2a), soon followed by a period of repetitive activity lasting for about  $2\frac{1}{2}$  min at both the PV and the right atrium. Another train of repetitive activity followed after a brief pause (Fig. 2b).

Closer examination of the record revealed that the extrasystoles were of PV origin triggered by the sinus beats. In normal conditions, action potentials at the right atrium always preceded those at the PV<sup>5</sup>. The first pair of action potentials of each train of repetitive activity was also led by the atrial muscle, indicating the sinus origin of these action potentials. However, the lead was shifted to the PV in all subsequent action potentials of the train, suggesting the diversion of the pacemaking site to a region close to the PV (Fig. 2c). These action potentials at the PV were characterized by large diastolic depolarizations. In contrast, action potentials at the right atrium arose abruptly from the baseline membrane potential. Subthreshold OAPs were observed at the PV but not at atrial sites after termination of each train. Figure 2d also demonstrates the initiation of a second train of repetitive activity triggered by the sinus beat and the subsequent shift of pacemaking site to the PV.

The amplitude of action potentials at the PV decreased gradually with time in ouabain. This decrease was more marked during repetitive activity. For example, the amplitude of action potentials decreased by about 30 mV at the end of the long train in Fig. 2b and remained small subsequently. There was also a slight decrement in the amplitude of action potentials at the right atrium during repetitive activity.

The duration of each period of repetitive activity decreased drastically within 10 to 20 min after their appearance. For example, 5 min after the long train shown in Fig. 2b, only three action potentials appeared in each train (Fig. 3a). This further

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decreased to two action potentials after a further  $2 \min (\text{Fig. } 3b)$ , and  $4 \min |\text{later}$ , no extrasystoles were observed (Fig. 3c).

To demonstrate that extrasystoles originated at the PV and required a triggering input, the PV was stimulated electrically using a pair of platinum wire electrodes placed close to the recording site. It was found that repetitive activity could also be initiated with electrical stimulation (Figs 3b, 4). Figure 3b demonstrates a coupled beat in response to a single stimulus at the PV. This response was similar to those occurring spontaneously except the phase lead of both the first and second pair of action potentials were now at the PV.

Conduction block between the PV and the right atrium developed in four out of seven preparations in the presence of ouabain. Figure 4a shows an example of unidirectional block with triggered activity at the PV coupled to the sinus beats. Repetitive activity at the PV did not affect atrial activity. A more severe block is demonstrated in Fig. 4b, in which the PV was quiescent and uncoupled to atrial activity. A strong electrical pulse applied at the PV proximal to the heart elicited an action potential in the atrium and at the same time triggered repetitive activity at the PV. After severing the PV from the heart, repetitive activity could still be triggered at the PV with electrical stimulation (Fig. 4c).

I have demonstrated here that the PV can act as an ectopic focus in atrial arrhythmia with digitalis intoxication. Thus, similar to Purkinje fibres, OAPs and repetitive activity developed at the PV in the presence of ouabain, the duration of repetitive activity decreasing with time of exposure to ouabain.

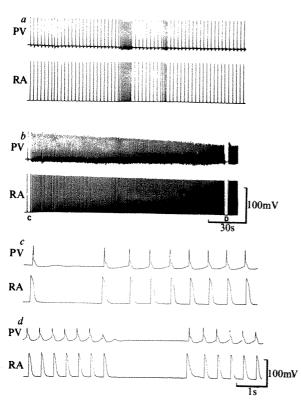


Fig. 2 Effect of a high concentration of ouabain  $(2.5 \,\mu\text{M})$  on the spontaneous activity of an atrium-PV preparation. After 75 min in ouabain, OAPs at the PV reached threshold. Initially, only two brief bursts were observed (a), but was followed soon after by a long train of repetitive response lasting for  $2\frac{1}{2}\min(b)$ . The first pair of action potentials of the train was characterized by the action potential at the RA preceding that of the PV (c). The phase lead then shifted to the PV in all subsequent action potentials of the train. These triggered action potentials were associated with pacemaking potentials at the PV. Subthreshold OAPs were observed at the PV following termination of each train (d). d Also shows the initiation of a second train by a sinus beat and the subsequent shift in phase lead to the PV. There was also a dramatic reduction in the amplitude of action potentials at the PV with repetitive activity (b).

The amplitude of action potentials of the PV also decreased dramatically with repetitive activity although the membrane potential did not alter significantly. A possible cause for the decrease in amplitude of action potentials and OAPs with prolonged exposure to ouabain is the accumulation of intracellular sodium. High intracellular sodium would shift the sodium

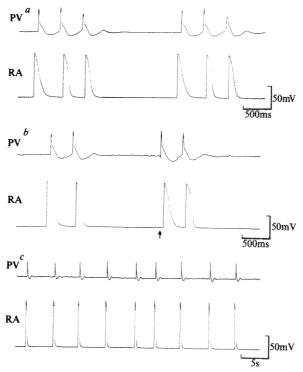


Fig. 3 The duration of repetitive activity decreased with prolonged exposure to ouabain. a Was recorded 5 min after the long train in Fig. 2b and b was recorded another 2 min later. Extrasystoles were not observed with further exposure to ouabain c. In b, an electrical stimulus (arrow) was applied close to the recording electrode at the PV to produce a coupled response similar to that occurring spontaneously.

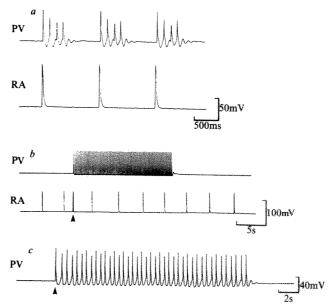


Fig. 4 Conduction block between PV and RA in the presence of ouabain (2.5  $\mu$ M). a, In this preparation, repetitive activity at the PV was coupled to the sinus beats. b, The PV was not coupled to the RA and was electrically quiescent. A single electrical stimulus applied at the PV close to the heart elicited repetitive activity at the PV and an action potential at the RA. In both cases, repetitive activity at the PV did not propagate into the RA. c, After being severed from the heart, repetitive activity could still be generated at the PV with electrical stimulation.

equilibrium potential, leading to a reduction in the amplitude of action potentials. The amplitude of OAPs might also be reduced if the transient inward current responsible for OAPs was also sodium dependent9. The antagonistic effect of high intracellular sodium on OAP may in fact provide a mechanism for spontaneous termination of repetitive activity. With high frequency firing during repetitive activity, the amplitude of OAPs would eventually decrease to below threshold because of the additional influx of sodium with the action potentials. In the brief interval between trains of repetitive activity, the concentration of intracellular sodium may decrease, depending on the degree of poisoning of the pump, and OAPs may again reach threshold for repetitive activity.

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#### Autoradiographic localization of GABA<sub>R</sub> receptors in rat cerebellum

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 $\gamma\text{-}Aminobutyric$  acid (GABA) is a major inhibitory neurotransmitter in the mammalian cerebellum  $^{1-4}.$  Evidence for the role of GABA in this brain region stems not only from neuropharmacological studies but also from in vitro and ex vivo ligand binding studies with 3H-GABA or 3H-muscimol5-10. The cerebellar concentration of GABA binding sites is higher than that elsewhere 11,12 even though it contains less endogenous GABA than many other brain regions<sup>13</sup>. Using an autoradiographic technique, Kuhar and co-workers<sup>14</sup> have recently demonstrated that these binding sites in the rat cerebellum (detected in Tris-citrate buffer) are located primarily in the granule cell layer, a finding which supports the observations of Candy and Martin<sup>15</sup> and Olsen and Mikoshiba<sup>8</sup> that <sup>3</sup>H-GABA binding is four to six times greater in homogenates of the granule cell layer than in the molecular layer. The GABA sites detected in these studies were bicuculline sensitive and exhibited a high affinity for muscimol. We would therefore classify them as GABAA sites because our recent observations have indicated the presence of other GABA sites (GABA<sub>B</sub> sites) in the central nervous system which are insensitive to bicuculline  $^{16-18}$ . These sites have a high affinity for  $\beta$ -p-chlorophenyl GABA (baclofen), the GABA analogue which is devoid of activity at GABAA sites. We describe here the autoradiographic localization of these GABA<sub>B</sub> sites within the cerebellum and show that, unlike GABAA sites, they are confined almost exclusively to the molecular laver.

As our previous studies on GABA<sub>B</sub> site binding have been limited to membranes prepared from whole rat brain 18,19 first necessary to examine the regional distribution of these binding sites before studying their cellular location within any

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one region. GABA<sub>B</sub> sites can be readily detected in crude synaptic membrane preparations using either <sup>3</sup>H-GABA (50 Ci mmol<sup>-1</sup>, Amersham) or <sup>3</sup>H-baclofen (8.8 Ci mmol<sup>-1</sup> Ciba-Geigy) as the ligand. It is essential, however, that the divalent cations  $Ca^{2+}$  or  $Mg^{2+}$  (1-5 mM) are present in the incubation medium and that Tris-HCl (50 mM, pH 7.4) is used instead of Tris-citrate buffer. Citrate presumably chelates any available divalent ions to prevent binding. High concentrations of Tris will also reduce GABA<sub>B</sub> site binding. In their autoradiographical studies, Palacios et al.14 used 0.3 M Tris buffer to detect GABA binding sites. At this concentration of Tris we found that whereas the GABAA site binding of <sup>3</sup>H-GABA (30 nM) is unaffected, binding to GABA  $_{\! B}$  sites is 57% lower than in 50 mM Tris. Consequently, the incubation and washing solution used for slices in the present study was 50 mM Tris-HCl buffer containing 190 mM sucrose (2.5 mM CaCl<sub>2</sub> was added to the solution used for GABA<sub>B</sub> site binding but was absent in GABA<sub>A</sub> site experiments). This isotonic solution (270 mosmol l<sup>-1</sup>) did not depress GABA<sub>B</sub> site binding and maintained the morphological integrity of the tissue slices for autoradiography.

For the initial distribution studies, separated brain regions were homogenized in 15 volumes of ice-cold sucrose (0.32 M) and pellets obtained as indicated in Table 1 legend, stored at 20 °C and used for assay within 3 days. The pellets were then thawed, washed four times and incubated with fixed concentrations of <sup>3</sup>H-GABA (10 nM) or <sup>3</sup>H-baclofen (20 nM). These are equivalent concentrations of active ligand, as <sup>3</sup>H-baclofen is a racemate and the (-)-isomer is >100 times more potent than the (+)-isomer as an agonist at GABA<sub>B</sub> sites.

Binding to GABA<sub>B</sub> sites was evident in all the brain regions studied and there was a striking correlation between the

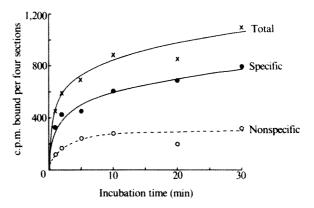


Fig. 1 Time course of <sup>3</sup>H-GABA binding to GABA<sub>B</sub> sites in cerebellar slices. Serial sections of a cerebellum were obtained from a male CSE rat anaesthetized with pentobarbitone before intracardiac perfusion with 0.1% paraformaldehyde in 0.01 M phosphate buffered saline (pH 7.4). The cerebellum was hemisected and immersed in isopentane at -40 °C. Sagittal sections (10 µm) were mounted on glass slides (four per slide) and allowed to dry at ambient temperature for 1 h before storage at -15 °C for at least 16 h. For the binding assay slides were removed from storage for 45 min before immersion in 250 ml Tris-HCl (50 mM, pH 7.4) containing CaCl<sub>2</sub> (2.5 mM) and sucrose (190 mM) for a further 50 min. Each slide was then air dried for 10-15 min after the removal of any excess fluid. Incubation solution (100  $\mu$ l) containing  ${}^3H\text{-}GABA$  (50 nM) and isoguvacine (40  $\mu$ M, to suppress Ca<sup>2+</sup>-independent binding to GABA<sub>A</sub> sites) with (O) or without (×) 100 μM (±)baclofen was applied to each slide and left for 1, 2, 5, 10, 20 or 30 min. The slides were then shaken separately to remove excess incubation solution and rinsed twice very briefly in fresh radioactive-free solution. After drying, the slides were cut and placed in scintillation vials with 0.4 ml distilled water. Scintillation fluid (10 ml Packard ES 299) was added to each vial 30 min later. The c.p.m. values are means of three determinations at each incubation time. Nonspecific values were those obtained in the presence of unlabelled baclofen. The specific values ( ) are the differences between total and nonspecific binding values determined at each incubation time.

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Table 1 GABA receptor distribution in rat brain

	В:	A site		
Brain region	<sup>3</sup> H-backofen (20 nM) (fmol per	<sup>3</sup> H-GABA (10 nM) mg protein)	(10 nM) (fmol per mg protein)	
Cerebellum Cerebral cortex Brain stem Spinal cord Mid-brain+ forebrain	76.1±12.5 22.7±5.5 13.9±4.3 11.4±2.6 23.4±4.0	$73.9 \pm 5.9$ $28.7 \pm 2.1$ $13.1 \pm 2.4$ $10.7 \pm 0.8$ $18.4 \pm 4.6$	$331 \pm 45$ $66.5 \pm 6.9$ $39.8 \pm 7.6$ $31.8 \pm 6.8$ $51.2 \pm 9.4$	

The regional distribution of GABA binding sites in rat brain membrane preparations. In each experiment separated brain regions from two rats were homogenized in 15 vol ice-cold sucrose (0.32 M) using a Potter-Elvjehem homogenizer fitted with a Teflon pestle. The homogenates were centrifuged at 1,000g for 10 min and the supernatants recentrifuged at 20,000g for 20 min. The pellets were then resuspended in the original volume of distilled water before centrifuging at 48,000 g for 20 min. The final pellets were frozen at -15 °C for at least 16 h. For the assay each pellet was allowed to thaw at room temperature for 30-40 min before resuspending and washing four times in 6 ml Tris-HCl buffer (pH 7.4, 50 mM) containing CaCl<sub>2</sub> (and isoguracine  $40\,\mu\text{M}$  to suppress binding to GABA<sub>A</sub> sites) for GABA<sub>B</sub> site or Tris-citrate buffer (pH 7.4, 50 mM) for GABA<sub>A</sub> site binding. The final pellet was resuspended in either medium (1-1.5 mg protein per 0.8 ml) solution determined by the method of Lowry et al. 22) for the assay. To each 0.8-ml aliquot 0.1 ml of incubation fluid with or without 1 mM ( $\pm$ ) backofen (GABA<sub>B</sub> site) or 1 mM isoguvacine (GABA<sub>A</sub> sites) was added. A further 0.1 ml containing <sup>3</sup>H-backofen or <sup>3</sup>H-GABA was added to provide final concentrations of 20 nM or 10 nM, respectively. Each mixture was incubated for 10 min at 20 °C and then centrifuged at 7,000g for 10 min. The supernatant was aspirated off and the pellet blotted dry before the addition of 100 µl Soluene and subsequent scintillation fluid. The data are from six separate experiments in each of which binding to GABAA and GABAB sites was determined simultaneously. Values are mean ± s.e.

amounts of <sup>3</sup>H-GABA and <sup>3</sup>H-baclofen specifically bound in each region. The concentration of GABA<sub>A</sub> sites was clearly much greater than that of GABA<sub>B</sub> sites in all regions, with an overall ratio of GABA<sub>A</sub>: GABA<sub>B</sub> sites of 3 or 4:1. The most important finding was the high concentration of GABA<sub>B</sub> sites in the cerebellum, which prompted us to examine the binding in this brain region more closely using an autoradiographic technique.

Brains from rats anaesthetized with pentobarbitone sodium (60 mg per kg intraperitoneally) were fixed by intracardiac perfusion with 0.1% paraformaldehyde in phosphate-buffered saline (0.01 M pH 7.4, 10 min perfusion with 200 ml). In some experiments higher fixative concentrations were used to see whether this affected the subsequent binding. At concentrations below 0.5% paraformaldehyde saturable binding was readily detected and apparently independent of the fixative concentration whereas above this concentration (up to 4%) it was markedly reduced and extremely variable. Sagittal slices (10 µm thick) of cerebellum were obtained by cryostat sectioning and four slices placed on single glass slides. The slices were allowed to dry at ambient temperature for 1 h before freezing at -20 °C. The tissue was stored in this manner for up to 1 week without any noticeable impairment in its ability to bind <sup>3</sup>H-GABA to either GABA<sub>A</sub> or GABA<sub>B</sub> sites.

3H-GABA (50 Ci mmol<sup>-1</sup>) rather than 3H-baclofen was used

<sup>3</sup>H-GABA (50 Ci mmol<sup>-1</sup>) rather than <sup>3</sup>H-baclofen was used for the experiments because it has a higher specific activity than <sup>3</sup>H-baclofen and no inactive enantiomer. Qualitatively similar results were obtained with <sup>3</sup>H-baclofen binding to GABA<sub>B</sub> sites but, as expected, the background radioactivity was higher and the total radioabelling lower than with <sup>3</sup>H-GABA.

For the binding experiments the slices were allowed to thaw and dry for 45 min and then washed for 50 min by immersion in incubation solution (250 ml per 30 slides). After drying, 0.1 ml incubation solution containing 40 nM <sup>3</sup>H-GABA with or

without unlabelled displacing agents was applied to each slide. This was subsequently removed by shaking and two brief rinses in large volumes of the appropriate Tris-sucrose solution. The slices were then either placed in a vial for scintillation spectrometry or kept for autoradiography (see Fig. 3 legend).

Figure 1 shows the time course of  ${}^{3}\text{H-GABA}$  binding to GABA<sub>B</sub> sites in these conditions. The sections were incubated with  ${}^{3}\text{H-GABA}$  in the presence of 40  $\mu$ M isoguvacine (to prevent GABA<sub>A</sub> site binding) with or without unlabelled ( $\pm$ )baclofen (100  $\mu$ M). The total amount of  ${}^{3}\text{H-GABA}$  bound increased with time for at least 20 min, whereas in the presence of unlabelled baclofen the amount bound (nonspecific binding) was constant after 5 min. The specific portion of binding (total minus nonspecific) remained constant after 20 min incubation and >85% of this had occurred by 15 min. All subsequent studies were performed using incubations of 15 min, by which time the specific portion comprised 75.4±2.3% (mean±s.e.m., n=9; triplicate determinations) of total binding. In the same conditions  ${}^{3}\text{H-GABA}$  binding to GABA<sub>A</sub> sites formed 90.1±1.6% (n=4 in triplicate) of the total binding.

Scatchard analysis of specific  $^3$ H-GABA binding to GABA<sub>B</sub> sites in cerebellar slices indicated the presence of a single saturable binding component  $K_d = 159$  nM,  $B_{\rm max} = 83.6$  fmol per 4 slices (Hill coefficient = 0.97). The apparent affinity of this component was therefore slightly lower in slices than previously observed in homogenates ( $K_d = 77$  nM), perhaps reflecting the presence of endogenous inhibitors in the intact slices which may be removed in the homogenate preparations.

<sup>3</sup>H-GABA which was specifically bound to GABA<sub>B</sub> sites in slices could be displaced by analogues shown previously to be

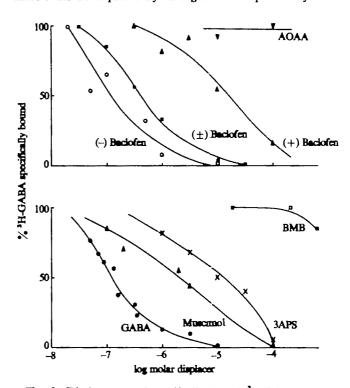


Fig. 2 Displacement of specifically bound <sup>3</sup>H-GABA from GABA<sub>B</sub> sites in rat cerebellar slices by unlabelled GABA-related substances. Cerebellar slices were obtained and treated in the same manner as described in Fig. 1 legend except that all slices were incubated for 15 min and various concentrations of different unlabelled analogues were added to the incubation solution. Total specific binding in any experiment was that portion of <sup>3</sup>H-GABA displaced by 100 μM (±)baclofen. The displacement produced by each concentration of analogue is expressed as a percentage of that occurring with (±)baclofen (100 μM). The data have been separated for clarity. Each value is the mean of three determinations (four slices per determination). Standard errors were <5%, ○, (-)Baclofen; Ⅲ, (±)baclofen; ▲, (+)baclofen; ▼, aminooxyacetic acid; ⊕, GABA; △, muscimol; ×, 3-aminopropanesulphonic acid; □, bicuculline methobromide.

active at GABA<sub>B</sub> sites in homogenates as well as whole tissue assays. Slices were incubated simultaneously with  $^3$ H-GABA and various concentrations of unlabelled displacer (as well as 40  $\mu$ M isoguvacine) and the combined data from these experiments are shown in Fig. 2. GABA and (–)baclofen were equipotent in displacing  $^3$ H-GABA, with IC<sub>50</sub> values of 120 nM. The IC<sub>50</sub> values for the other analogues were 400 nM ( $\pm$ )baclofen, 2  $\mu$ M for muscimol, 12.6  $\mu$ M for 3-aminopropanesulphonic acid and 15.8  $\mu$ M for (+)baclofen, an order of potency which accords with the analogue specificity observed previously in homogenates  $^{16}$ . As expected, bicuculline metho-

bromide  $(100 \, \mu M)$  and the GABA transaminase inhibitor aminoxyacetic acid (AOAA) were inactive. In the latter case, as both GABA and AOAA would be expected to bind to the active site of the enzyme, the absence of effect of AOAA suggests that the transaminase enzyme is unlikely to be the binding site in slices.

Inhibitors of GABA transport—cis-3-aminocyclohexane carboxylic acid (ACHC), nipecotic acid and 2,4 diaminobutyric acid (DABA)—produced no displacement at  $10~\mu M$  and less than 20% displacement at  $100~\mu M$ ; the IC<sub>50</sub> values were all  $\gg 1~mM$ . It is therefore unlikely that the binding site is a trans-

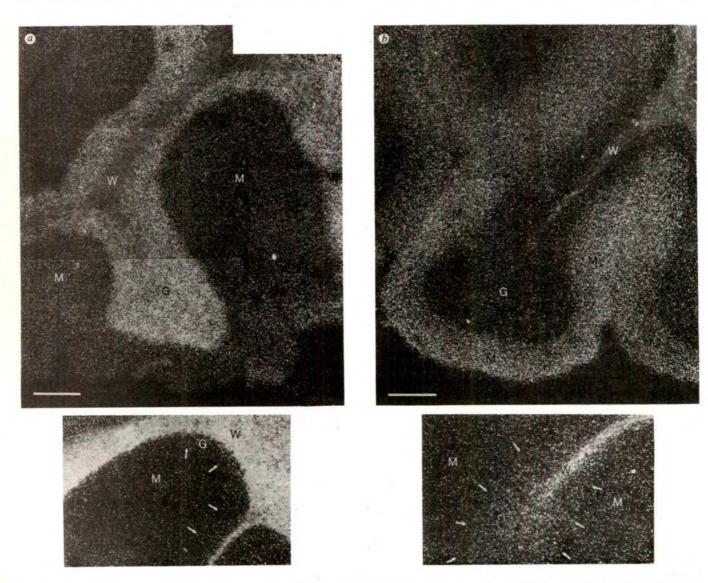


Fig. 3 Light microscopic darkfield autoradiograms of <sup>3</sup>H-GABA binding to GABA<sub>A</sub> sites (a) and GABA<sub>B</sub> sites (b) in rat cerebellum. Cerebellar cryostat sections were obtained as described in Fig. 1 legend. After thawing and drying for 45 min the sections were immersed in Tris-HCl solution containing sucrose (190 mM) with (GABA<sub>B</sub> sites) or without (GABA<sub>A</sub> sites) CaCl<sub>2</sub> (2.5 mM) for 50 min. For GABA<sub>A</sub> site binding, 100 µl of incubation fluid containing 3H-GABA (50 nM) with (nonspecific binding) or without (specific binding) unlabelled isoguvacine (100 µM) was applied for 15 min. This was removed by shaking and two brief rinses in large volumes of incubation medium. For GABAB site binding the same procedure was followed except that the incubation medium contained CaCl2 (2.5 mM) and isoguvacine (40 µM) to suppress binding to GABA<sub>A</sub> sites. Nonspecific binding was determined by the addition of  $(\pm)$ baclofen  $(100 \,\mu\text{M})$ . After drying, the slides were prepared for autoradiography following the procedure of Young and Kuhar<sup>23</sup>. Coverslips that had been dipped in Ilford K5 emulsion (emulsion : water 1:1.5) were attached to the slides with Loctite glue and batches of six slides were held together with bulldog clips and exposed for up to 12 days at +4°C in a sealed box containing silica gel. Slides were developed at 16°C in Kodak 19b developer (3.5 min) and fixed in 25% sodium thiosulphate solution. Following several washes in glass distilled water the slides were dried overnight and the coverslips were then permanently fixed in position with DPX mounting medium. Sections were viewed under dark field optics with a Leitz Ortholux II microscope and photographs taken with Ilford FP4 film. GABAA sites (a): The greatest concentration of silver grains can be seen over the granule cell layer (G) and a lesser concentration over the molecular layer (M). Very few grains are present over the white matter tract (W). The smaller photograph below shows nonspecific binding. The boundary between the molecular and granule cell layers is indicated by the arrows. GABA<sub>B</sub> sites (b): In contrast to a, the greatest accumulation of silver grains is over the molecular layer (M) with a lesser amount over the granule cell layer (G) and white matter (W). In the section below, displaying nonspecific binding, the boundary of the molecular and granule cell layer is again delineated by arrows. The white matter tracts in such sections show varying degrees of light scattering; here it is greater in the blank (lower picture) than in the experimental section. Such light scattering, however, does not hinder the counting of silver grains. Scale bar, 300 µm.

Table 2 Autoradiographic grain densities over cerebellar layers following 3H-GABA binding to GABA, GABA, and both sites together in 10-µm cryostat sections

	GABA <sub>B</sub> sites			GABA <sub>A</sub> sites		GABA <sub>B</sub> and GABA <sub>A</sub> sites  Total -			
Layer	Total	Nonspecific	Total – nonspecific	Total	Nonspecific	Total nonspecific	Total	Nonspecific	nonspecific
White matter Granule cell layer Molecular layer	1.21±0.16 1.64±0.17 3.82±0.17	$1.08 \pm 0.07$ $1.12 \pm 0.08$ $1.09 \pm 0.05$	0.13 0.52 2.73	$1.44\pm0.12$ $9.88\pm0.62$ $3.85\pm0.18$	$0.87 \pm 0.08$ $0.94 \pm 0.08$ $1.10 \pm 0.19$	0.57 8.94 2.75	1.24±0.36 10.47±0.83 6.38±1.06	$0.76\pm0.06$ $0.85\pm0.05$ $0.95\pm0.05$	0.48 9.62 5.43

Densities were quantified after 1 week's exposure using an eyepiece graticule. In each case the mean ±s.e.m. was obtained after counting four separate areas each of 5,400  $\mu m^2$  on two slides. Values are grains per 100  $\mu m^4$ .

port recognition site for GABA, and this is supported by the Na+ independence of binding in slices.

The close comparison between the pharmacological specificity observed in slices and homogenates indicated that it was appropriate to proceed with autoradiography in the slices. Thus, slides which had been incubated in <sup>3</sup>H-GABA to label GABAA or GABAB sites were dried, coverslips coated with emulsion (Ilford K5) were placed in contact with the sections, and the slides were stored in boxes at +4 °C for 6-12 days before developing. Figure 3 gives results from two slices from the same cerebellum after 12 days' exposure. Figure 3a shows the distribution of silver grains after incubation with <sup>3</sup>H-GABA (50 nM) in the absence of Ca2+ and presence of 100 µM (±)baclofen; the associated background picture was obtained by the further addition of isoguvacine (100 µM). Figure 3b shows the distribution after incubation with <sup>3</sup>H-GABA in the presence of 2.5 mM CaCl<sub>2</sub> and 40 μM isoguvacine; (±)baclofen (100 μM) was further added to obtain the background picture. The distribution of silver grains in the two incubation conditions was markedly different. GABA, site binding (Fig. 3a) occurred primarily in the granule cell layer whereas GABA<sub>B</sub> site binding (Fig. 3b) was confined to the molecular layer. The grain counts for these individual layers are shown in Table 2.

Concomitant experiments using slices from the same animal were subjected to analysis by scintillation spectrometry. The mean radioactivity (c.p.m. values per four slices,  $\pm$ s.e.m., n = 6) for GABA<sub>A</sub> and GABA<sub>B</sub> sites, respectively, were 3,591±269 (background  $393\pm156$ ) and  $962\pm3$  (background  $236\pm66$ ). The specific portions were therefore 3,198 and 726 c.p.m., respectively, which represents a ratio of 4.4:1. When slices were incubated in <sup>3</sup>H-GABA in the presence of Ca<sup>2+</sup> alone to obtain the total GABA, and GABA, site binding, the value was  $4,203 \pm 303$  c.p.m. (background  $236 \pm 34$ ). The specific portion was then 3,967 c.p.m., which was identical with the sum of the

separate data  $(3,198\pm726=3,924 \text{ c.p.m.})$ . The present study has shown that  $Ca^{2+}$ -dependent GABA binding sites (GABA<sub>B</sub> sites) in the rat cerebellum are localized almost entirely within the molecular layer. Whether these sites are functional remains to be seen, although it may be pertinent that whereas the number of presumed GABA nerve terminals is similar in the molecular and granule cell layer, 'classical' GABA, sites are unequally distributed14. The presence of GABA<sub>B</sub> sites may explain this apparent deficit of GABA

receptors in the molecular layer.

We do not know the cell type(s) on which the GABA<sub>B</sub> sites are located. Evidence from release studies has suggested that they are present on presynaptic terminals where they modulate transmitter release 16,20. However, we know of no morphological evidence for axo-axonic synapses on terminal boutons in the molecular layer21. Thus, allowing for the fact that such a population may not have been recognized, other possibilities are that GABA<sub>B</sub> sites are (1) non-innervated (2) present postsynaptically on morphologically 'conventional' axo-dendritic synapses or (3) present on glial cells. This third possibility seems unlikely because there is no autoradiographic evidence of binding over fibrous astrocytes present in white matter, and pellets enriched in synaptic membranes show a high degree of binding. Work is in progress to investigate these possibilities.

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#### Neurotensin receptors are located on dopaminecontaining neurones in rat midbrain

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Neurotensin is a putative peptide neurotransmitter in the mammalian brain; neurotensin-containing neurones neurotensin receptors have been identified in the brain both blochemically and histochemically<sup>1-7</sup>. There is strong evidence for an interaction between dopaminergic systems and neurotensin<sup>7-11</sup>. Related to this, light microscopic radiohistochemical studies have shown a dense localization of neurotensia receptors in the substantia nigra zona compacta and the adjacent ventral tegmental area of the rat midhrain. These studies suggest a high concentration of neurotensin receptors on dopaminergic cell bodies. Here we report a large, local depletion of neurotensin receptors in the substantia nigra zona compacta after a local 6-hydroxydopamine injection, indicating that endogenous neurotensin or related drugs could have potent effects on dopaminergic function in the brain.

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Male, Sprague-Dawley rats (225-275 g) were used in our experiments. 6-Hydroxydopamine (Sigma) was dissolved in saline containing 0.2 mg ml-1 ascorbate to give a final concentration of 2 mg ml-1 and 6 µg of drug were injected over a 5 min period with a Hamilton 5 µl syringe, which was placed stereotaxically over the substantia nigra as previously described12 Three animals were injected with drug and three were injected with saline as controls. Four weeks after injection, the animals were killed and tissues prepared for receptor autoradiography as previously described<sup>7,13,14</sup>. Sections (8 μm) of midbrain were thaw-mounted on to microscope slides and neurotensin receptors labelled by incubating the sections in 4 nM <sup>3</sup>H-neurotensin (64.3 Ci mmol<sup>-1</sup>; New England Corporation) in 0.17 M Tris-HCl buffer pH 7.6 at ice-bath temperatures. The buffer contained 0.05% boiled bovine serum albumin (BSA) and 0.1 ml bacitracin. After incubation, the sections were washed twice for 5 min in the same buffer at 2 °C to reduce nonspecific binding. Blank values were obtained by adding 5 µM neurotensin tmparallel incubations. Extensive biochemical studies have shown that binding of neurotensin to the slide-mounted tissue sections in these conditions is predominantly to the neurotensin receptor<sup>7</sup>. Autoradiographs, produced by the apposition of <sup>3</sup>H-Ultrofilm (LKB) in X-ray film cassettes<sup>14</sup>, were exposed for 30 days at 2 °C. After development, the autoradiographic grain densities were determined quantitatively using a micro-

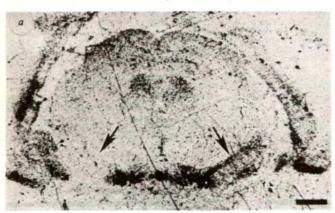






Fig. 1 Autoradiographic localization of neurotensin receptors to dopaminergic neurones in rat substantia nigra zona compacta. Injection of 6-hydroxydopamine results in a loss of neurotensin receptors and dopamine-containing cells in the substantia nigra zona compacta (see text for details). a, Photomicrograph of the autoradiographic image of the rat midbrain on <sup>3</sup>H-Ultrofilm. Note the loss of radioactive ligand binding on the left (at arrow) where the 6-hydroxydopamine was injected, compared with the binding on the right where receptor densities parallel the distribution of dopamine-containing cells. Scale bar, 1 mm. b, c, Higher power photomicrographs of the cresyl violet-stained section used to produce the image in a. The photomicrographs were taken at the arrows in a. Note the loss of cell bodies in the zona compacta in b (corresponding to the injected left side in a) compared with the opposite uninjected side, shown in c. Scale bar, 100 μm.

Table 1 6-Hydroxydopamine injection in the rat substantia nigra affects neurotensin receptor binding

Brain area	Neurotensin receptor binding (fmol per mg protein)				
	Control side	Lesion side	% Change		
Substantia nigra					
(pars compacta)	$128.3 \pm 25.8$	$12.3 \pm 7.9$	-90 (P < 0.01)		
Ventral					
tegmental area	$197.7 \pm 25.9$	$226.3 \pm 21.7$	+15*		
Rhinal sulcus	$175.2 \pm 13.3$	$177.0 \pm 20.4$	+1*		
Cortical nucleus of					
the amygdala	$161.1 \pm 19.0$	$162.3 \pm 28.3$	+1*		

Data are mean ± s.e.m. of at least two tissue sections from each of three different animals. Data were obtained by measuring the optical densities of the autoradiograms as described in the text. By using autoradiographic standards, the optical densities were converted to fmol of ligand bound. Blank values of receptor binding were quite low and have been subtracted. Thus, the data represent specific receptor binding.

\* No significant difference, Student's t-test.

densitometer (Gamma Scientific) with a 100-µm aperture. Autoradiographic standards were included in each experiment so that grain densities could be transformed to fmol of neurotensin bound.

In these rat midbrain sections, the receptor distribution was identical to that previously reported<sup>7</sup>. High densities were found in the substantia nigra as well as in the adjacent ventral tegmental area. Neuronal elements containing immunoreactive neurotensin have been detected in this area<sup>3</sup>. High densities of receptors were also found in the cortical nucleus of the amygdala, rhinal sulcus and in parts of the cerebral cortex. Serial, adjacentsections incubated with excess neurotensin to provide blanks, did not produce any significant elevated grain densities in hese areas.

Injection of 6-hydroxydopamine caused a reproducible and substantial loss of cell bodies in the substantia nigra zona compacta. There was also a striking loss of neurotensin receptors in the substantia nigra that did not extend into the medial ventral tegmental areas (Fig. 1). No loss of receptor occurred in the saline-injected controls (data not shown). Thus, there was receptor loss only in areas showing cell destruction. Biochemical studies indicated a 75–80% reductionin dopamine levels in the ipsilateral striatum confirming a loss of dopamine cells in the injected substantia nigra (data not shown). There was no nonspecific loss of cells at the injection sites as benzodiazepine receptor levels were not decreased substantially in adjacent serial sections (data not shown).

To quantitate the changes in receptor distributions, the autoradiographic grain density was determined by microdensitometry (Table 1). There was a 90% loss of neurotensin receptors on the injected side of the substantia nigra. By contrast, areas unaffected by the lesion, such as the ventil tegmental area, rhinal sulcus and cortical nuclus of the amygdala, did not show any significant change in receptor density.

These results indicate that at least some dopamine-containing neurones in the ventral midbrain of the rat have very high densities of neurotensin receptors. This is corroborated by the recent finding of Andrade and Aghajanian<sup>15</sup> that neurotensin is a highly potent and reliable excitant of dopaminergic neurones in iontophoretic studies. Our results, taken together with those of others<sup>7-11,15</sup>, agree with the general idea of a neurotensin-dopamine interaction in brain, probably at several sites<sup>3,7-11</sup>. Dopaminergic neurones are presumably affected byother peptides including enkephalins<sup>16</sup>, cholecystokinin<sup>17</sup> and substance P<sup>18</sup>. Thus, the development of peptide agonists and antagonists may be useful in the treatment of syndromes involving dopaminergic neurones.

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#### Isolation and biogenesis of a new peptide from pancreatic islets

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Pancreatic polypeptide (PP) was discovered as a major peptide contaminant of insulin preparations<sup>1,2</sup>. The peptide is stored in a distinct endocrine cell type<sup>3</sup> that is the predominating islet cell in the duodenal part of the pancreas<sup>4</sup> and it is released in response to physiological events, including food intake<sup>5</sup> and decrease in blood glucose<sup>6,7</sup>. Furthermore, infusions of PP mimicking physiological fluctuations in plasma concentrations of the peptide affect gastrointestinal functions, by inhibiting exocrine pancreatic secretion<sup>8-10</sup> and relaxing the gallbladder<sup>10,11</sup>. and relaxing the gallbladder1 Although the PP cell is more sensitive to small reductions in blood glucose than is the glucagon cell<sup>6</sup>, many studies have failed to demonstrate any metabolic effect of PP<sup>12,13</sup>. It is recognized that the biogenesis of secretory peptides by conversion of larger precursors results in multiple peptide fragments of which more than one might be bioactive 14-16. Recently we identified a precursor of PP with a molecular weight (M,) of 8,000-10,000 and showed, by pulse-chase experiments and peptide mapping, that this biosynthetically labelled precursor was converted into a low molecular weight peptide in addition to PP17. We report here the isolation and primary structure of this icosapeptide and show how it arises through an intermediate form by post-translational modification of the COOH-terminal part of the PP precursor. This second product of pro-pancreatic polypeptide conversion might fulfill the physiological potential of the major endocrine cell type of the duodenal pancreas.

To obtain amounts of the co-synthesized pro-PP-related product sufficient for structural, immunological, and biological studies, we used the biosynthetically labelled peptide as a probe. Duodenal lobes of canine pancreas which are especially rich in PP<sup>18</sup> were extracted in acid ethanol and gel-filtered. As shown in Fig. 1a, a peak of absorbance (280 nm) eluted at the position of the biosynthetically labelled fragment of the PP precursor (fractions 125-135). This peak was not obvious when extracts were prepared from the splenic part of the pancreas, which is deficient in PP (data not shown). Only one major peptide eluting with the labelled fragment of the PP precursor was detected by polyacrylamide gel electrophoresis (Fig. 1a inset). (The proinsulin connecting peptide (C-peptide) which elutes in a similar position neither absorbs at 280 nm nor is it fixed and stained in the polyacrylamide gel system used.) The peptide was further

purified by DEAE-anion-exchange chromatography (Fig. 1b) and by paper electrophoresis using polyacrylamide gel electrophoresis as a monitoring system. The amino acid composition of the purified peptide, determined after acid hydrolysis using an amino acid analyser (Dionex) is as follows: Asp, 2.08; Ser, 0.86; Glu, 2.02; Pro, 1.81; Gly, 2.01; Ala, 2.91; Met, 0.68; Ile, 0.95; Leu, 1.05; His, 1.04; Arg, 3.02. The peptide had an absorbance at 280 nm compatible with the presence of one tryptophan residue, giving a total composition of 20 amino acids. Aspartic acid was determined as the NH2-terminal residue by identification of the corresponding dansyl derivative on polyamide sheets (A 1700; Schleicher & Schuell) after treatment with dansyl chloride. The primary structure of the icosapeptide was determined by manual Edman degradation and by degradation with carboxypeptidase Y; the sequence and details of the methods used are given in Fig. 2 legend.

Both chemical and immunological experiments were performed to determine the structural relationship between the isolated icosapeptide and the biosynthetically labelled material

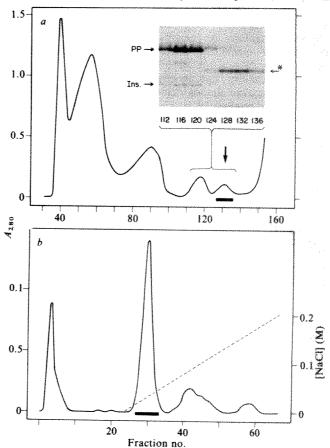


Fig. 1 Purification of the new peptide arising from pro-PP conversion. Duodenal lobes of pancreas (~20 g each) were excised from anaesthetized mongrel dogs and immediately homogenized in ice-cold acidified ethanol (0.1 M HCl in 68% ethanol, final concentrations). The homogenate was incubated overnight at 4 °C and centrifuged (40,000g, 25 min). The supernatant was neutralized and the peptides partially purified by ether precipitation<sup>27</sup>. a, Gel filtration profile of proteins extracted from four pancreases on a 2.5×175 cm Bio-Gel P-30 column eluted with 3 M acetic acid. The vertical arrow corresponds to the elution position of the biosynthetically labelled fragment of the PP precursor (peptide III) obtained as described in Fig. 3 legend. The inset shows Amido black-stained peptides after polyacrylamide slab gel electrophoresis (10% acrylamide, pH 8.7; ref. 28) of aliquots from selected fractions of the area shown. The positions of PP and insulin (Ins) standards in the gels are indicated. The asterisk indicates the position of the peptide which was followed throughout the purification. The solid bar shows the fractions which were pooled and evaporated for further purification. b, Elution profile of gel-filtered peptides on DEAE-Sephadex. The ion-exchange column  $(1 \times 4 \text{ cm})$  was equilibrated and eluted at  $4^{\circ}$ C with 0.02 M Tris buffer pH 8.4 containing 6.5 M urea and with a NaCl gradient of 0-0.2 M (400 ml total volume). The solid bar indicates the fractions which were pooled and desalted over Bio-Gel P-2 using 3 M acetic acid. The peptide was further purified by paper electrophoresis on Whatman 1 or 3 paper in 30% formic acid and was cluted from the paper with 88% formic acid at 4 °C

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identified in PP cells. Endocrine cells were isolated from canine duodenal pancreas as previously described<sup>17</sup> and purified on a Percoll gradient as described in Fig. 3 legend. The cells were incubated with <sup>35</sup>S-methionine or with <sup>35</sup>S-methionine plus <sup>3</sup>H-leucine and the biosynthetically labelled peptides were studied initially by gel filtration. Three major radiolabelled peptides were detected (Fig. 3a). Peptide I is a biosynthetic precursor of PP which is converted during pulse-chase experiments into PP (peptide II) and its co-synthesized product (peptide III)<sup>17</sup>. The elution profile of labelled peptides from these freshly isolated, single cells was similar to that previously obtained from cultured, aggregated cells ('pseudoislets').

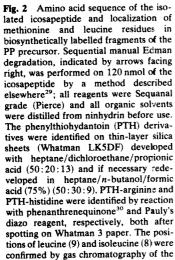
The biosynthetically labelled peptides identified in Fig. 3 were immunoprecipitated with anti-PP antibodies as well as antibodies raised against the icosapeptide (see Fig. 3 legend). The middle profile in Fig. 3a shows that the radiolabelled PP and its radiolabelled precursor are both precipitated by immune complexes prepared with anti-PP antiserum. The lower profile in Fig. 3a demonstrates that the radiolabelled PP precursor and the co-synthesized conversion product, peak III, are both absorbed specifically to immune complexes prepared using antibodies against the newly isolated icosapeptide. Thus, peptide I is specifically immunoprecipitated by antibodies against both PP and the icosapeptide, which shows that the precursor contains immunochemical determinants of both peptides. We have already demonstrated by peptide mapping that tryptic fragments of peptides II and III co-migrate with those of peptide I<sup>17</sup>. These two lines of evidence identify both the structure of the new icosapeptide and that of PP as occurring in the same peptide precursor.

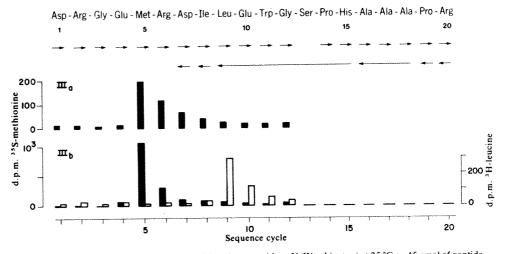
The radiolabelled material in peak III (which was quantitatively immunoprecipitable by antibodies raised against the icosapeptide) separated into two peptides during polyacrylamide gel electrophoresis; one of these co-migrated with the icosapeptide whereas the other migrated more towards the anode at pH 8.7. To study the biosynthetic relationship between these peptides, the radiolabelled peptides obtained from peak III during a pulse-chase experiment were subsequently examined by polyacrylamide gel electrophoresis. As shown in Fig. 3b, upper profile, radioactivity appeared initially in the more anionic peptide, IIIa, during the pulse period. The middle and lower profiles in Fig. 3b demonstrate that during continued incubation in the presence of unlabelled amino acid, the radioactivity in the anionic peptide IIIa first increased (in

agreement with the processing of the precursor during the first 75 min of the pulse period<sup>17</sup>) and eventually decreased, while the radioactivity in the icosapeptide, peptide IIIb, increased. This shift in proportion of radioactivity between the two peptides, both of which are immunoprecipitable by antibodies against the icosapeptide, is consistent with the more anionic peptide being an intermediate in the biogenesis of the icosapeptide, the final product.

To determine the structural relationship between the two peptides eluting in peak III, they were purified by gel filtration and polyacrylamide gel electrophoresis and subjected to sequential Edman degradation. As shown in Fig. 2, <sup>35</sup>S-methionine was identified at position 5 in both peptides; in addition, <sup>3</sup>H-leucine was identified at position 9 in the peptide which co-migrated with the icosapeptide. (Only peptide IIIb originated from a double label experiment.) The positions of these labelled amino acid residues align with the methionine and leucine residues in the NH<sub>2</sub>-terminal sequence of the isolated icosapeptide. The identical position of the methionine residue in the NH<sub>2</sub>-terminal sequence of the two radiolabelled peptides indicates that they differ at their COOH-terminus and that the intermediate form bears a short COOH-terminal extension to the icosapeptide sequence.

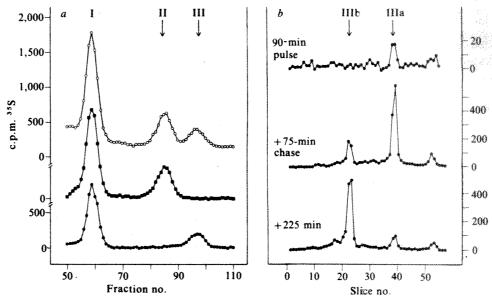
As the pancreatic polypeptide sequence has been localized at the NH<sub>2</sub>-terminus of peptide I<sup>17</sup>, the results shown in Figs 2 and 3 indicate that the icosapeptide originates from the COOHterminal part of the common precursor, from which the icosapeptide sequence is excised as an intermediate form (see Fig. 4); subsequent proteolytic events then refine the COOH-terminal region of the intermediate and result in the mature icosapeptide. The fact that the COOH-terminal residue of the icosapeptide is arginine suggests that an enzyme with a trypsin-like specificity is involved in cleavage of the intermediate form. Converting enzymes for peptide precursors usually require a dibasic amino acid sequence and often remove completely the converting-site residues. However, COOH-terminal arginyl residues are found in epidermal growth factor 19 and nerve growth factor 20, both of which result from precursor cleavage by arginyl peptidases<sup>21,22</sup> In addition, the first two residues (Asp-Arg) are identical to the NH2-terminal residues of angiotensin, which form the site for the specific conversion of angiotensins to des-Asp<sup>1</sup>-angiotensin, angiotensin III<sup>23</sup>. The icosapeptide derived from the processing of the PP precursor may be susceptible to a similar proteolytic modification.





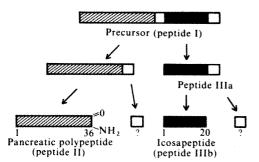
corresponding PTH derivatives. The COOH-terminal sequence was studied by degradation with carboxypeptidase-Y (Worthington) at 25 °C on 45 nmol of peptide with a molar enzyme/peptide ratio of 1:100 in 0.1 M N-ethylmorpholine brought to pH 7.0 with acetic acid. Norleucine (25 nmol) was added for standardization and aliquots removed after the following intervals: 0, 0.5, 1, 3, 5, 10 and 60 min, and 20 h. Free amino acid content was determined using an amino acid analyser. The arrows facing left indicate the amino acid residues which were placed in sequence from the time course of appearance during digestion with carboxypeptidase-Y; long arrows indicate that several amino acids were released too closely to be placed in sequence. No amino acyl phenylthiohydantoin derivative was identified after the arrows indicate that several amino acids were release of serine during carboxypeptidase digestion, as shown, is consistent with a serine at position 13. The 13th cycle of Edman degradation. However, the release of <sup>35</sup>S-methionine (closed columns) and <sup>3</sup>H-leucine (open columns) during manual Edman degradation histograms in the lower part of the figure illustrate the release of <sup>35</sup>S-methionine (closed columns) and <sup>3</sup>H-leucine (open columns) during manual Edman degradation of the biosynthetically labelled PP precursor fragments (peptides IIIa and IIIb) obtained as described in Fig. 3 legend. These peptides were purified by polyacrylamide gel electrophoresis (15% acrylamide) at pH 8.7 (ref. 28) and by elution from the gel slices into 88% formic acid; the peptides were desalted over Bio-Gel P-2 using 3 M acetic acid. The icosapeptide (50 nmol) was used as carrier during both the purification and sequence analysis.

Fig. 3 Biosynthesis of PP and the icosapeptide in isolated endocrine pancreatic cells. Single cells were prepared from duodenal, canine pancreas by trypsin digestion in Ca<sup>2+</sup>-free medium as previously described<sup>17</sup>. The endocrine cells were purified on a Percoll gradient and cells floating to the interface between layers having densities of 1.047 and 1.060 were used. The cells were incubated at 37°C in Eagle's basal medium (with Hanks' salts) lacking methionine, but containing 0.2 mCi <sup>35</sup>S-methionine</sup> containing 0.2 mCi <sup>35</sup>S-methionine (890 Ci nmol<sup>-1</sup>; Amersham). After 4 h, the cells were centrifuged and washed twice before extraction in 1 ml of 3 M acetic acid. The extract was then applied to a 1.5 × 90 cm Bio-Gel P-30 column and the column eluted with 3 M acetic acid 10 mg l<sup>-1</sup> bovine albumin (BSA). a, Elution profiles of peptides appearing in the 12,000–1,000  $M_r$  region. Aliquots from the column fractions were either counted directly or precipitated with preformed



immune complexes prepared against PP or against the isolated icosapeptide as described previously<sup>17</sup>. (Anti-bovine PP antiserum (146-7) was given by Dr R. E. Chance, Lilly Research Laboratories, Indianapolis). Antibodies against the icosapeptide (isolated as indicated in Fig. 1) were raised in a rabbit (3202-2) injected subcutaneously with the peptide coupled through its  $\alpha$ -amino group by difluorodinitrobenzene<sup>31</sup> to BSA. Total radioactivity ( $\bigcirc$ ), radioactivity specifically immunoprecipitated with PP-antibody complexes ( $\blacksquare$ ) and radioactivity specifically immunoprecipitated with icosapeptide-antibody complexes ( $\blacksquare$ ) are shown. Specific immunoprecipitated radioactivity is defined as the difference in radioactivity precipitated in the absence and presence of excess immunocompetitor (bovine PP<sub>1-36</sub> and highly purified icosapeptide, respectively). The void volume of the column corresponds to fraction 31 and PP has an elution position corresponding to peak II. b, Polyacrylamide gel electrophoresis profiles of labelled peptides obtained from peak III during a pulse-chase experiment. Endocrine cells were incubated with 3S-methionine for 90 min (pulse period, top panel) followed by incubation with excess unlabelled methionine for 75 min (middle panel) or 225 min (bottom panel). The radiolabelled peptides were extracted and purified by gel filtration as described for a and peptides eluting in peak III were pooled and subjected to polyacrylamide gel electrophoresis in 8 cm-long gels containing 15% acrylamide. The gels were sectioned into 1.5 mm slices and peptides were eluted into 0.5-ml portions of 88% formic acid. The isolated icosapeptide co-migrated with peptide IIIb in this system; the tracking dye bromophenol blue migrated to slice 53.

Although many large precursor molecules have been identified, only a few cases of the biogenesis of more than one stable peptide product from a single precursor have been documented. A classic example is the biosynthesis of insulin and C-peptide from proinsulin and the co-secretion of the two peptide products<sup>14</sup>. However, the C-peptide apparently serves only as a connector of the two chains of the insulin molecule. The secretion of equimolar amounts of glucagon and a fragment of the NH<sub>2</sub>-terminal part of the glucagon precursor has also been described<sup>16</sup>, but very little is known about the structure of this molecule and its possible function. Proopiomelanocortin, a precursor which contains the sequence of several bioactive peptides such as adrenocorticotropic hormone (ACTH), melanocyte stimulating hormone (MSH), and  $\beta$ -endorphin<sup>15,3</sup> is a more complex example. Post-translational modification of proopiomelanocortin seems to differ between cell types and thus regulates the appearance of the different co-secreted peptides. for example, acetylation of  $\alpha$ -NH<sub>2</sub> groups will activate  $\alpha$ -MSH whilst inactivating the endorphins<sup>25</sup>. Here we have reported the structure of the icosapeptide fragment of the PP precursor, which is stored in similar amounts to PP in the duodenal pancreas. Further studies will establish whether the storage and secretion of the icosapeptide or a related form can solve the



Scheme for the organization of pancreatic polypeptide and icosapeptide within the common precursor and for the conversion of the precursor to its final stored products; PP and the icosapeptide are indicated by hatched and solid bars, respectively. The primary structure of PP has previously been localized to the NH<sub>2</sub>-terminus of the precursor by radiosequencing<sup>17</sup>. Roman numerals refer to the peptide designations used in Figs 2, 3

physiological enigma concerning the function of the PPcontaining cells in the pancreas as well as in other tissues, for example the nervous system.2

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### Action of glutamate and aspartate analogues on rod horizontal and bipolar cells

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Electrophysiological evidence indicates that transmitter release from retinal rod terminals occurs at a high rate in the dark and is reduced by light<sup>1-6</sup>. The rod transmitter closes ionic channels (mainly sodium channels) in the subsynaptic membrane of a class of bipolar cells (on-centre bipolar cells) which depolarize in response to light<sup>4,7-9</sup>. However, the transmitter opens sodium channels in the subsynaptic membrane of horizontal cells4 We report here that several compounds, chemically related to the amino acids aspartate and glutamate, have similar postsynaptic actions to that of the rod neurotransmitter. Kainic acid<sup>11</sup>, in micromolar concentration, hyperpolarizes rod oncentre bipolar cells, increasing their membrane resistance while depolarizing rod horizontal cells. Experiments with 2-amino-4phosphonobutyric acid (APB), in which a phosphono group is substituted for a carboxyl group of glutamic acid, have further distinguished between different binding sites on the cells which make synaptic contact with rods. APB is an agonist at the on-centre bipolar cells, closing the same ionic channels as the rod neurotransmitter, but is without effect on rod horizontal cells. The evidence presented here suggest that the widely held view that glutamate or aspartate is the rod neurotransmitter needs to be re-examined.

Intracellular recordings were made in on-centre bipolar cells and horizontal cells of the virtually all-rod retina of the dogfish,  $Scyliorhinus\ canicula$ . Sectors of the dark-adapted eyecup were superfused with oxygenated elasmobranch Ringer's solution at pH7.8 and  $\sim 16$  °C. Equilibration time, after switching perfusion fluids, was several minutes, limited by diffusion through the vitreous and retina. Test flashes of blue-green light, 15 ms in duration, were applied periodically. Full-field illumination was used. Methods of intracellular recording, identification of cells, resistance measurements and light calibration have been described previously.

The effect of kainate on horizontal cells and on-centre bipolar cells is illustrated by typical records in Fig. 1a and b. Figure 1a shows a horizontal cell, depolarized by kainate at a concentration of 2  $\mu$ M, the membrane potential increasing to +6 mV in the presence of 20 µM kainate. The upper trace (recorded simultaneously) shows the progressive decrease in the hyperpolarizing response to light of the horizontal cell with increasing concentration of kainate, the response to bright flashes being almost completely abolished by 20 µM kainate. On removal of kainate, the cell hyperpolarized to a final internal potential of -64 mV, close to its initial value in the dark (shown at the beginning of the lower trace); and, as seen in the upper trace, responses to light also recovered. The effects of kainate on horizontal cells are consistent with an action at the same sites as the rod neurotransmitter but do not rule out the possibility that the effect is exerted at a site presynaptic to the horizontal cell or at extrasynaptic sites on the horizontal cell. However, in other experiments synaptic activity was blocked by 2 mM Co<sup>2+</sup>, making the former possibility unlikely. The addition of Co2+ resulted in hyperpolarization of horizontal cells, to an internal potential between -100 and -120 mV, which was the same as the potential to which the cell could normally be driven by bright

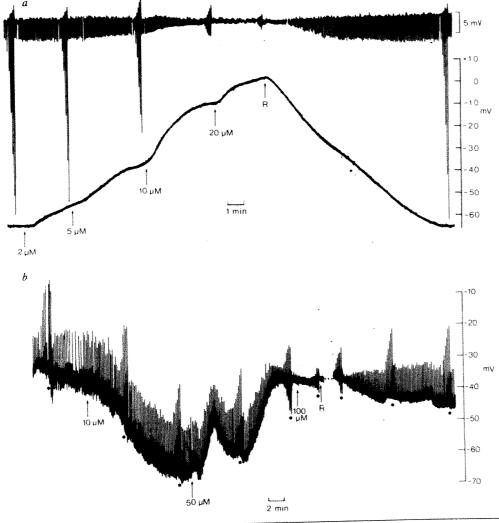


Fig. 1 The action of kainic acid on the membrane potential and response to light of a horizontal (a) and an on-centre bipolar cell (b). In this and succeeding figures, test flashes of constant intensity were applied every 5-10 s; at intervals, the cell's light intensityresponse relation (which appears as a staircase in response) was determined by increasing the light intensity by factors of ~2. a, The upper record shows the response of a rod horizontal cell to flashes, the response being represented as a displacement from the d.c. level in the dark. A sample-and-hold circuit was used to centre the d.c. level about an arbitrary zero before each flash<sup>9</sup>. The lower simultaneous record (at lower gain) shows the d.c. potential of the horizontal cell. Each of the constant test flashes bleached 0.87 rhodopsin molecules per rod (=0.87 Rh\*\*). The brightest flash during each of the intensity-response determinations bleached 67 Rh\*\*. Perfusion with normal Ringer's solution is indicated by R. Normal elasmobranch Ringer's solution contained 280 mM NaCl. 3 mM KCl, 4 mM CaCl<sub>2</sub>, 3 mM NaHCO<sub>3</sub>, 0.5 mM MgCl<sub>2</sub>, 350 mM urea, 10 mM glucose and 5 mM HEPES buffer pH 7.8. b Shows the effect of different concentrations of kainic acid on the membrane potential and response to light flashes of a bipolar cell. Test flashes, each bleaching 0.024 Rh\*\*, were applied throughout the record except when the light intensityresponse relation was determined. Closed circles indicate the brightest flash of the series, bleaching 1.8 Rh\*\* line indicates a break of 15 min in the record.

light, which presumably turns off transmitter release. The addition of  $50-100~\mu M$  kainate resulted in depolarization to an internal potential of about zero, indicating that kainate acts directly on horizontal cells.

In contrast to its action on horizontal cells, low concentrations of kainate (<10 μM) hyperpolarized on-centre bipolar cells. As shown in Fig. 1b, following the addition of 10 µM kainate to normal Ringer's solution, the bipolar cell hyperpolarized by 29 mV to an internal potential of -67 mV. The hyperpolarization was associated with an increase in input resistance from an initial value of  $26 \,\mathrm{M}\Omega$  to  $50 \,\mathrm{M}\Omega$ . After switching to higher concentrations of kainate, from  $50 \mu M$  to  $100 \mu M$ , complex transient effects on on-centre bipolar cells were observed, and responses to light flashes were significantly reduced. It is likely that complex effects on bipolar cells arise from a dual action of kainate, one a direct effect on the bipolar cell, the other arising from depolarization of horizontal cells which interact with bipolar cells. Figure 2 shows evidence that the interaction between horizontal and on-centre bipolar cells involves a synaptic mechanism. When synaptic transmission was blocked by Co<sup>2+</sup>, 100 µM kainate monotonically hyperpolarized the on-centre bipolar cells, driving the membrane potential to values more negative than its normal value in the dark (Fig. 2a). Figure 2b shows the voltage displacements produced by applied current before, during  $\mathrm{Co}^{2+}$  application and during application of Co2+ plus 100 µM kainate. Block of transmitter release by Co<sup>2+</sup> depolarized the bipolar cell by 11 mV to a value of the internal potential which was slightly less than the maximum potential to which it could be driven by bright light. The input resistance decreased to 24 M $\Omega$  from its initial value of 39 M $\Omega$ . During the steady-state hyperpolarization by 100 µM kainate, the input resistance increased to 42 M $\Omega$ . The reversal potential for transmitter action was -15 mV, estimated from the intersection of the linear portion of current-voltage relations in normal Ringer's and during Co2+ block of transmitter release9. These results are consistent with the idea that the rod neurotransmitter closes ionic channels which are relatively selective for Na+. The reversal potential for kainate action, estimated from the intersection of the current-voltage relations during Co<sup>2+</sup> block in the presence or absence of kainate, was estimated to be -13 mV. This result indicates that kainate also closes ionic channels which are relatively selective for Na+. The difference in values of reversal potential are within the error of the method.

The data may also be used to compare the changes in membrane potential with the resistance changes. If the channels closed by kainate and rod neurotransmitter had the same reversal potential, any change in resistance should be proportional to the change in membrane potential, but of opposite sign for a substance which closes channels. This was found to be the case on the assumption that the resistance measurements were not more accurate than  $\pm 5\%$ . It was not possible in these experiments to distinguish whether the effect of kainate was at synaptic or extrasynaptic sites of on-centre bipolar cells. However, extrasynaptic open channels would constitute a shunt on the normal response to light and would reduce synaptic gain on-centre bipolar cells.

The putative transmitters, L-glutamate and L-aspartate, had similar effects on rod horizontal and on-centre bipolar cells to those of kainate, but were at least three orders of magnitude less potent, glutamate being about three times more active than aspartate. The required concentration of glutamate or aspartate was several orders of magnitude greater than expected for a neurotransmitter. As aspartate and glutamate may be transported into the cells and metabolized, the concentration at synaptic sites may be less than in the perfusate. Thus, concentration alone is not sufficient evidence against glutamate or aspartate being the rod neurotransmitter.

To examine further the possibility that glutamate or aspartate could be the rod neurotransmitter, substances which act as antagonists to these putative transmitters elsewhere in the nervous system were tested. One of these was APB, which binds

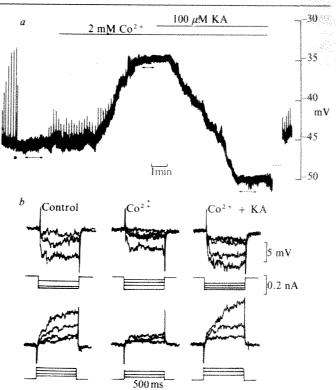


Fig. 2 Effect of kainic acid on membrane potential and input resistance of an on-centre bipolar cell when synaptic transmission has been blocked by Co<sup>2+</sup>. a, Record of the membrane potential and response to 15-ms flashes given at 10-s intervals. At the beginning of the record, the control response to light flashes, increasing in intensity by factors of  $\sim 2$ , is shown. The brightest bleached 4.4 Rh\*\*. The intervals indicated by arrows show the time when resistance measurements were made. The voltage displacements produced by the current pulses have been removed from the record for clarity. Low-intensity test flashes bleaching 0.053 Rh\*\* were applied throughout the rest of the recording. A 15-min/break in the record following the removal of Co<sup>2+</sup> and kainic acid is shown by a dotted line. b, Voltage displacements produced by hyperpolarizing and depolarizing current pulses of 500 ms duration, applied during the intervals shown in a. A single microelectrode was used for applying current and recording voltage. The voltage drop across the resistance in series with the cell's capacitance was balanced by a bridge circuit. The voltage displacement produced 100 ms after current onset, was used in constructing current-voltage curves. Because the records were noisy, the voltage displacements at 90-110 ms at a given applied current were averaged from six to eight records. The input resistance was estimated from the slope of the current-voltage relation in its linear region (displacements of ±5 mV). Some of the apparent nonlinearity in the records is due to time-dependent changes in the resistance of the electrode during current flow. Cell input resistance in the absence of  $Co^{2+}$  and kainic acid (control) was 39 M $\Omega$ ; with 2 mM  $Co^{2+}$ , 24 M $\Omega$ ; with  $Co^{2+}$ plus 100  $\mu M$  kainic acid, 42  $M\Omega$ .

competitively to glutamate-binding sites 13 and acts as a competitive antagonist to glutamate 13,14 at synapses where there is good evidence that glutamate is the excitatory transmitter 15,16 As shown in Fig. 3a, 50 µM APB reversibly hyperpolarized on-centre bipolar cells and blocked their responses to light, despite the increase in driving force for the light response; however, APB had no significant effect on horizontal cells (Fig. 3b). The experiment, illustrated in Fig. 4, in which synaptic transmission was blocked by  $Co^{2+}$  shows that the hyperpolarization of on-centre bipolar cells by APB results from its direct action on the bipolar cell. The increase in input resistance is evidence that APB acts by closing ionic channels. The reversal potential for transmitter action and APB action, estimated from the current-voltage relations, was the same, -31 mV for the cell illustrated. From these results we conclude that APB acts on the same sites of on-centre bipolar cells as those which are controlled by light. The selectivity of its action is due to different binding sites for the rod neurotransmitter on horizontal and on-centre bipolar cells.

APB is similar in size and charge distribution to glutamic acid and is  $\sim 500$  times more effective than glutamate on on-centre bipolar cells. It is interesting that 2-amino-3-phosphonoproprionic acid, which is shorter in length by one carbon

atom (and is the phosphono-analogue of aspartic acid), is also without effect on horizontal cells and is 50-fold less potent than APB on on-centre bipolar cells. The analogue, 2-amino-5phosphonovaleric acid (APV), in which the chain length is increased by one carbon atom, had no effect on on-centre bipolar cells at a concentration of 0.1 mM (at least 20 times the concentration required for a detectable effect of APB). APV has been reported to be a potent antagonist of the excitatory effect of aspartate and glutamate in the mammalian and frog spinal cord<sup>17</sup>. The chemically related compound, D- $\alpha$ -aminoadipate (DαAA) also acts as an antagonist to the excitatory actions of aspartate, and to some extent glutamate, on neurones of the central nervous system  $^{18-20}$ . We found that 5 mM D $\alpha$ AA had no effect on membrane potential or responses to light of on-centre bipolar cells. The racemic mixture (1 mM DLaAA) was also tested on rod horizontal cells and likewise was without effect. This is in contrast to the reported action of DLa AA on horizontal cells with an exclusive cone input, which provided some evidence for aspartate as a cone neurotransmitter<sup>21</sup>. Interestingly, the cone transmitter opens K+ or Cl- channels of oncentre bipolar cells in the retina of teleost fish whereas the rod transmitter closes Na+ channels of the same bipolar cells8.

In view of the finding that glutamate and aspartate are only effective in considerably higher concentrations than required of APB and kainate, and that none of the antagonists tested here showed effects consistent with their expected actions, we suggest

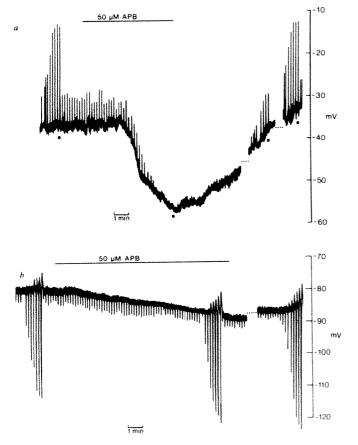


Fig. 3 Selective action of 2-amino 4-phosphonobutyric acid (APB) on on-centre bipolar cells compared with horizontal cells. a, The membrane potential and response to flashes of a bipolar cell. Closed circles indicate when the brightest of the test flashes was used in obtaining the relation between light intensity and response (4.4 Rh\*\* except in the presence of APB when the brightest flash was 8 Rh\*\*). The test flashes used throughout the rest of the record had an intensity of 0.024 Rh\*\*. The first break in the record after washing out APB lasted 3 min, the second 11 min (dotted lines). b, The membrane potential and response to flashes of a horizontal cell. The small hyperpolarization seen after the addition of APB is probably due to a better seal of the electrode in the cell and not due to drug action, as it was not observed in other experiments. The brightest of the flashes in each of the intensity-response series was 136 Rh\*\*. Each of the low-intensity test flashes bleached 0.47 Rh\*\* except after the break in the record lasting 18 min, when the intensity was 0.2 Rh\*\*

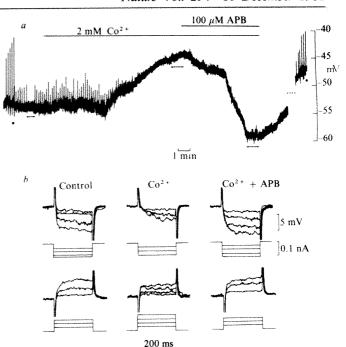


Fig. 4 Effect of APB on membrane potential and input resistance of an on-centre bipolar cell after block of synaptic transmission by Co2+ Membrane potential and response to test flashes. The brightest flash at the beginning and end of the record (shown by closed circles) was of intensity 5.5 Rh\*\* Throughout the rest of the record. except during the intervals 5.5 Rh\*\*. Throughout the rest of the record, except during the intervals shown by arrows, the test flash was of intensity 0.029 Rh\*\*. Resistance measurements were made during the arrowed intervals. The dotted line indicates a break of 12 min. b, Voltage displacements produced by 200-ms hyperpolarizing and depolarizing current pulses. Input resistance estimated in the way described in Fig. 2 legend: control, 42 M $\Omega$ ; with 2 mM Co<sup>2+</sup> 25 M $\Omega$ ; with Co<sup>2+</sup> plus 100  $\mu$ M APB, 54 M $\Omega$ .

that the candidacy of glutamate and aspartate as rod neurotransmitters requires re-examination.

The results raise the possibility that the rod transmitter acts at the same sites of rod horizontal and on-centre bipolar cells as those on which kainate acts. There is evidence that kainate may act at sites which are different from glutamate sites 22-24. That the sites of transmitter binding on rod horizontal cells must be distinct from those of on-centre bipolar cells is shown by the selective action of APB. A similar selective effect of APB on on-centre bipolar cells of the mixed rod-cone retina of the mudpuppy has recently been described<sup>25</sup>.

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The Systematics Association Special Volume No 19

# Biosystematics of Social Insects

edited by P.E. Howse and J.-L. Clement

September 1981, xii + 346pp., £28.20 (UK only)/\$68.00, 0.12.357180.4

This volume contains papers by eminent international specialists on social insects submited at a symposium organized by the International Union for the Study of Social Insects in conjuction with the Systematics Association and held in Paris in September 1980. The symposium was devoted to the evaluation of different techniques and the comparison of results. Entomologists, taxonomists and agronomists will all be interested in this important work. In addition, the methodologies and techniques described here will interest all zoologists concerned with the problems of systematics.

# Haemophilus, Pasteurella and Actinobacillus

Proceedings of an International Symposium, August 1980, Cophenhagen, Denmark. edited by M. Kilian, W. Frederiksen and E.L. Biberstein.

October 1981, xiv + 294pp., £14.20 (UK only)/\$29.50, 0.12.406780.8

This book attempts, for the first time, a joint discussion of these three genera of closely related bacteria. It includes reviews of their history, ecology, physiology, and clinical importance in human and veterinary medicine. Results of the application of new methods in taxonomy such as studies of DNA homology, respiratory quinones, enzyme patterns, and electrophoretic analysis of cellular proteins are presented. Collectively, information acquired from these studies allows a more precise definition of the three genera and their species and facilitates the separation of Haemophilus, Pasteurella and Actinobacillus from neighbouring taxa.

# Engineering Calculation Methods for Turbulent Flow

Peter Bradshaw, Tuncer Cebeci and James H. Whitelaw

March 1981, xii + 332pp., £18.60 (UK only)/\$45.00, 0.12.124550.1 Contents: Conservation equations and boundary conditions. Turbulence models based on eddy viscosity hypotheses. Stress equation modelling. Introduction to numerical methods for two-dimensional and steady, three-dimensional flows. Computer program for unsteady two-dimensional boundary layers. Recirculating flows. Viscous inviscid interactions and corner flows. Stability and transition. Wings. Turbomachinery. Combustion.

# Fluorescent Probes

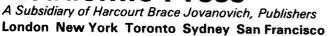
edited by G.S. Beddard and M.A. West

July 1981, x+236pp., £15.20 (UK only)/\$36.50, 0.12.084680.2

The papers collected in this volume were first presented at a meeting, 'Fluorescence Probes in Proteins and Membranes', held at the Royal Institution of Great Britain, in November 1979. Many have been updated by their authors since the meeting. Papers covering the latest time dependent work indicate the value of these measurements for both intrinsic and extrinsic probes. The chemical properties of various site-specific probes are also discussed together with applications to energy transfer and lectin distribution in normal and malignant cells. The book includes a comprehensive bibliography of work in the area between 1970 and 1980.

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# **Academic Press**



# The Genetics and Biology of Drosophila

Volume 3a edited by M. Ashburner, H.L. Carson and J.N. Thompson Jr.

November 1981, xvi + 428pp., £40.00 (UK pniy)/\$96.00, 0.12.064945.4

This volume is founded on the remarkable advances made in evolutionary genetics. The first chapter of Volume 3a considers the world fauna. Subsequent chapters explore the geographical distribution, history and special features of the fauna of the Nearctic, Neotropical, Palearctic, Afrotropical, Oriental and Pacific Oceania regions, Australia and New Zealand. There is a chapter on domestic and widespread species and the book closes with a review of bizarre Drosophilidae to round out this broad taxonomic and geographical survey.

# Biology and Control of Take-all

edited by M.J.C. Asher and P.J. Shipton October 1981, xvi+452pp., £41.40 (UK only)/\$99.50, 0.12.065320.6

After a brief historical introduction the taxonomy and biology of the take-all fungus are reviewed comprehensively with reference to the relationship of the pathogen to closely-related species. The ecological, physiological and genetic factors that influence the development of the disease are discussed in some detail and the interaction between the pathogen and the host is examined. In the final section several aspects of take-all disease as it occurs under field conditions are considered.

**GIAM VI** 

# Global Impacts of Applied Microbiology

Sixth International Conference edited by S.O. Emejuaiwe, O. Ogunbi and S.O.

June 1981, viii + 652pp., £20.60 (UK only)/\$49.40, 0.12.238280.3 The Sixth International Conference on Global Impacts of Applied Microbiology (GIAM VI) was held in Lagos between 31st August and 6th September 1980. The proceedings of this conference, published here, not only sets forth the achievements of recent research but demonstrates a dramatic advancement towards the attainment of some of the practical goals of applied microbiology in the modern world: adequate food supply, good health and a clean environment.

Institute of Mathematics and Its Applications Conference Series

# The Mathematical Theory of the Dynamics of Biological Populations II

edited by R.W. Hiorns and D. Cooke

July 1981, xii + 328pp., £14.50 (UK only)/\$35.00, 0.12.348780.3
Based on a conference held in Oxford in July 1980, the book brings together leading mathematicians and biologists to review recent developments in the mathematical study of biological populations. Discussion is centred around two key subjects: the stability and control of populations, and the behaviour and structure of population. Biometrical aspects are also discussed. A worthy addition to the existing volume published in 1973, this work will enhance yet further the interface between mathematics and biology.

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Announcement and Call for Papers

# Diagnostic Histopathology

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#### AIMS AND SCOPE

This journal is an independent international periodical which is published quarterly. It aims to provide a forum for original papers concerned primarily with correlating structure and function in normal and pathological tissues.

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A. McNicol

IMMUNOSUPPRESSION WITH CYCLOSPORIN A: A Review C.J. Green

FETAL AND NEONATAL LIVER DISEASE D.I. Rushton

#### EDITORIAL

During the past 4 years the journal "Investigative and Cell Pathology" has attracted and published articles of a high scientific and medical standard which have helped both diagnostic pathologists and those with more scientific interest. Nonetheless, it has become clear that there is a need within the framework of histopathology for a further forum for the presentation of descriptive pathology, especially where functional aspects may be added to provide greater insight into disease processes. It is for this reason that the title of the journal has been changed to "Diagnostic Histopathology". It is hoped, that in doing so, a journal will be provided for histopathologists which they will use while making diagnostic assessments of debatable and

journal will be provided for histopathologists which they will use while making diagnostic assessments of debatable and difficult lesions. The journal will also aim to be one to which histopathologists refer to learn of current improvements in methods of diagnoses or understanding of disease. The high quality of illustrated material established in the journal will continue to be a feature of each publication. It is hoped that the journal will continue to appeal to the scientific as well as the diagnostic pathologist and that it will bridge the gap which is so important to the future improved understanding of disease processes.

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# **BOOK REVIEWS**

# **Biochemist extraordinary**

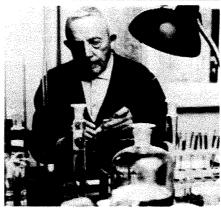
Hans Kornberg

BIOCHEMISTRY has always been, and is likely to remain, an empirical science: a discipline in which knowledge accrues by experiment only. It is therefore not unexpected that major advances in the understanding of biological processes at the molecular level often follow the development of novel experimental techniques. Such developments occur when methods, currently available, are no longer adequate to provide answers to important questions; they usually involve the application to biological problems of technological advances made in other and often unrelated areas of science.

It is rare that one person has the combination of abilities that enables him to distinguish problems that are both important and capable of being resolved successfully, and to initiate and develop the novel means of studying them. It is even rarer to find this combination allied to so ruthless and single-minded a purpose that, for virtually the whole of his adult life, that person subordinates all other activities to the pursuit of his scientific goals. Such a person was Otto Warburg.

As Sir Hans Krebs reminds us in this masterly little book (there are only 81 pages of text, with a further 60 of documentation), Warburg's scientific achievements were immense. In the 1920s he adapted the manometric techniques, developed by Haldane and Barcroft for measurement of the amounts of gases in various body fluids, so that they became the main means not only for analysing a wide range of metabolites but also for measuring the rates at which cellular and sub-cellular events proceed. Warburg's investigation of the structure of the ironcontaining component of the respiratory enzymes, that he had discovered by these techniques, led him to pioneer the use of photoelectric cells and monochromatic light sources to measure its action spectrum. This, in turn, led to the development of the UV spectrophotometer. Together with the Warburg manometer, the widespread use of this instrument permitted the pathways of cellular metabolism to be almost completely elucidated in the 1950s and 1960s. Incidentally, it was Warburg who discovered the identity of most of the coenzymes of respiration, including NAD, NADP, FMN and FAD; who discovered the change in light absorption at 340 nm consequent upon change in the oxidation state of NAD and NADP; and who isolated and purified numerous enzymes of carbohydrate metabolism including some - such as the first enzyme of the pentose

Otto Warburg: Cell Physiologist, Biochemist and Eccentric. By Hans Krebs. Pp.141. ISBN 0-19-858171-8. (Oxford University Press: 1981.) £10, \$24.95.



Otto Warburg at work, July 1966

phosphate pathway — not previously described. It is recorded that Warburg was judged worthy to receive a Nobel Prize on no fewer than three occasions over a period of nearly 20 years, for three separate scientific achievements.

But a price had to be paid for these successes. Throughout his astonishingly long and fruitful career, Warburg deliberately refused to "waste" time in pursuits that would not advance scientific discovery. Thus, apart from his interests in music and a wide knowledge of literature, Warburg shunned ties of family, of friendship or even of scientific fellowship; he did not teach, join committees or tolerate interruptions of his chosen routine. On one occasion, he refused an honorary DSc because he did not wish to devote time to collecting it. One would be tempted to see Warburg as an arrogant, self-righteous and monstrously egocentric despot in his laboratory, were it not for the thoughtful and sympathetic portrait that Sir Hans draws and that colours, with warmth and affection, this otherwise stark picture.

Sir Hans knew Warburg, as his student, colleague and (to some extent) confidant, for half a century. His accounts of the history and significance of Warburg's scientific work, and of the atmosphere in which it was carried out, make fascinating reading for any student of contemporary biology; his assessment of Warburg's

This review was written before the death of Sir Hans Krebs last month. His Reminiscences and Reflections, to be published in January, will be reviewed in Nature at a later date.

personality — his strengths, weaknesses, foibles, warts-and-all — makes equally absorbing reading for any student of human nature. One does not need to be an historian of science to feel a debt of gratitude to Sir Hans Krebs for producing what is as much an eminently readable work of scholarship as it is a labour of love.

Sir Hans Kornberg is Professor of Biochemistry at the University of Cambridge and a holder of the Otto Warburg Medal.

# **Creating confusion**

John D. Barrow

The Creation, By P.W. Atkins. Pp.132. ISBN 0-7167-1350-0. (W.H. Freeman: 1981.) £5.95, \$9.95.

THE memorable Dr Pangloss was introduced to the public by his creator in order to lampoon a traditional but cyclopean perception of the natural world — the prejudice that there exists some benign and covert principle of optimality governing all things. The style of *The Creation* suggests that Pangloss must have had descendants who are not only alive and well but actively popularizing modern science. Ironically, they are no longer theists, but materialists advocating the pursuit of "extreme reductionism and militant rationalism".

The aim of this little book — and it is a good deal smaller than one might think since the text only appears on every other page with footnotes on the alternates - is to convince the lay reader that everything is as it is because it simply could not have been any different. Rather than take the traditional view that some Creator is necessary to explain the existence and arrangement of things, or (the equally traditional view) that he is not, the author argues that by extrapolating contemporary cosmological knowledge we can support a scenario wherein the Deity himself is confronted with what is now an increasingly familiar problem - redundancy. Roughly speaking, it is argued that nature behaves as the ultimate totalitarian state in which everything that is not forbidden is compulsory. By examining the laws and foundation properties of nature (as we now see them), Atkins tries to argue that different universes are not possible and that the traditional craftsman Creator who carefully constructs universes by hand is replaced by the computerized machinery of mathematical necessity.

Suspicions that we might be dealing with a new religion here are confirmed when on p.7 we read that "the only faith we need for the journey is the belief that everything can be understood and ultimately there is nothing to explain". But, I suspect most readers' quarrel with the contents of Atkins's book would not be these aims or declarations of intent but the fact that the case he argues thereafter is also based upon faith or "knowledge" that usually does not vet exist. A Panglossian approach is followed whereby the author creates the type of future knowledge that would support his argument. This argument reminded me of mediaeval "proofs" for the existence of God, and suffers the same weakness that removed their ability to persuade. The disproof of any step in the arguments would not shake their authors' position one inch because the foundation of their belief really rested elsewhere.

That the bulk of the author's argument is speculation is not in itself a bad thing, but if we examine the way in which he handles known facts it ought to give us some guide as to the likelihood that this speculation is reliable. A few results of this rain-check were not too encouraging: he erroneously claims that it is the Lorentz signature of space-time that prevents time-travel; that the rate of biological evolution is exponentially sensitive to the fine structure constant (in fact, doubling the present value of the fine structure constant would, according to modern grand unified gauge theories, cause all protons to decay before stable stars form rather than increase the human evolution time to 1062 years!); and that the temperature of the environment is independent of the fine structure constant (it is actually determined by it).

To my mind, the author does not even get close to showing his case is a scientific possibility since his explanation for the inevitability of the creation of space-time ex nihilo requires an initial state of "almost nothing" — a dust of unstructured points (supplied by a well-known American relativist).

I am afraid that I would not recommend this book to the audience of laymen and students for whom it was created. Scientific popularizers and even scientific evangelists have a responsibility to wait until we know something about the creation of the Universe before telling the public everything about it. If you say, as the author does, that "complete knowledge is just within our grasp", to your professional colleagues it is quite harmless because they will simply laugh at you. To make such a statement in a popular book is totally misleading. This book will be far from enlightening and, I believe, will do little more than create confusion between the known, the unknown and the unknowable in the mind of the inquiring reader.

John Barrow is a Lecturer in Astronomy at the University of Sussex.

# The results of thinking small

Wolfgang K.H. Panofsky

The Nature of Matter. Edited by J.H. Mulvey. Pp.200. ISBN 0-19-8151151-5. (Clarendon/Oxford University Press: 1981.) £8.95, \$15.95.

GOOD popular expositions on particle physics are rare; this is one of them. It is a sequence of separate lectures given by prominent figures in the field at Wolfson College in 1980. In contrast to the common pattern in such series, the lecturers — at least occasionally — cross-refer to one another's talks, and there are other elements of coordination which make the volume rather better than most multiauthor works.

Sir Denys Wilkinson opens the book with an excellent overview, starting with the development of atomistic concepts and ending with the efforts at grand unification of forces and particles. The subsequent discussions of particles and forces are similar to traditional popular presentations of the subject. I found the lecture by Llewellyn-Smith on manifest and hidden symmetries particularly useful, since it gives a comprehensible account of what is more generally called symmetry breaking where the usual layman's exposition leaves the reader in doubt why a broken symmetry is a symmetry at all.

The section by Abdus Salam, on unification of forces, is a straightforward, logical exposition beginning with a historical summary of how the many apparently disjointed forces in nature became understandable in terms of a smaller and smaller set. He then goes into more detail on the history of the unification of the gauge theories of electrodynamics and the weak interaction. His lecture is nicely interwoven with anecdotes; at the same time I fear the reader will gain relatively little understanding of the intellectual content of the theory — this, however, would be a difficult job in any circumstances. The lecture ends with an enumeration of the problems faced in the formulation of a grand unified theory and the alternative approaches to a quantum theory of gravitation.

John Ellis has written a fascinating chapter on the very large and the very small, which is mainly an outline of the extent to which our growing knowledge of the reactions of particle physics limits cosmological models.

I was somewhat less impressed by the experimental sections, in part because they are very brief and overshadowed by the theoretical lectures. The lecture by D.H. Perkins, "Inside the Proton", attempts to give an overview of the quark structure of nucleons and provides the usual quark and lepton tables. The fundamental neutrino scattering experiments which are of particular interest to the lecturer are elaborated, but the balance of the evidence

for hadron structure is covered only briefly. As a result the reader may get a misleading impression that the foundation on which the current theories of hadron structure rest is quite limited, while actually a firm experimental basis exists.

I have a similar reservation about the section on the tools of particle physics. The author, Sir John Adams, concentrates on the history and technical realization of proton circular accelerators. Since he is the creator of the CERN proton synchrotron and super proton synchrotron, this emphasis is fully justified. Yet, on reading this chapter, the layman will underestimate the richness of the arsenal of tools available to the experimentalist, in terms of accelerators, colliders and detectors. In particular - and here my own parochial view is evident - the essential role of electron-positron colliders, which have reaped a substantial fraction of recent discoveries, does not come through. This apart, the lecture includes some illuminating remarks on the social patterns that physicists have established in using the expensive tools of modern particle physics.

As a whole, The Nature of Matter does provide a readable account of various topics in elementary particle physics, ranging over the enormous breadth of dimensions to which modern theory applies. Yet what the layman will miss in the book is the appreciation that understanding of the forces and components of nature depends largely on experiment. The lecturers take little account of the impact of the major experimental discoveries - remember, for instance, the upset when the mu meson turned out not to be the carrier of the strong interaction predicted by Yukawa. Let us recall, too, the many unsuccessful attempts to explain theoretically the experimentally observed tau-theta paradox until Lee and Yang hit upon the right solution; the impact of the cosmic ray discovery of the "hooks and forks" which then became identified with the strange particles, from which then flowed the quark models of Gell-Mann and Zweig; the discovery of the Charmonium peaks which initiated the November Revolution of 1974; and finally the discovery of the third charged lepton which again was not predicted.

Somehow more has to be done in popular accounts of particle physics so that advances in the field are not conveyed as a sequence of events where theorists predict the future, machine builders construct suitable machines, experimenters design instruments and experimental arrangements to test the theory, and then theory is tested. That is not the way it is.

Wolfgang K.H. Panofsky is Director of the Stanford Linear Accelerator Center, California.

# Live issues: the classification of North American mammals

Malcolm C. McKenna

The Mammals of North America, 2nd Edn. By E. Raymond Hall. Two volumes, pp.1,181. ISBN Vol. 1 0-471-05443-7; ISBN Vol. 2 0-471-05444-5. (Wiley: 1981.) \$59.85, £33.25 per volume; two-volume set \$106.40, £59.15.

E. RAYMOND Hall and Keith R. Kelson's compilation of the species-level taxonomy and geographical distribution of living North American mammals, published in 1959, has long been out of print. Now Hall has produced a partly corrected second edition, updated to 1977 and even beyond, but the new version is still marred by many jarring anachronisms.

As before, the work is divided into two volumes. After a brief introduction, the first volume begins with marsupials and then marches through the insectivores, bats, primates, edentates, lagomorphs and the first half of a long section on rodents. The second volume completes the rodents and then moves to the cetaceans, carnivores, sirenians, perissodactyls and artiodactyls. In all of the entries, attention is focused on the traditionally-studied details of coat colour and measurements of the body, limbs, ears and tail - the sort of information one needs when setting out to match a stuffed mouse with specimens in the neatly arranged rows of museum trays. Many illustrations and occasional discussions of skull morphology are also provided, although, for me, details of some dentitions are depicted at too small a scale. There are helpful indexes, a glossary and a long bibliography, and a "how to do it" section deals with collection of specimens, preparation and data preservation.

I believe that the work is still flawed by its typological, philatelic approach - the preoccupation with segregating on the basis of a few selected criteria, mainly Rubicons of size, what might as well be inanimate, history-bereft objects. Although dichotomous keys are employed frequently, these have little phylogenetic rationale and do not deal with morphocline polarities as would be the case if the organisms were treated cladistically. No attempt is made to weed out sharedprimitive characters. Worse, in the keys geographical distributions are all too often used in place of biological characters just as stratigraphical position does not necessarily yield the correct identification

The Biochemistry of the Nucleic Acids, originally written by J.N. Davidson and first published in 1950, has recently appeared in a ninth edition. The new edition has been compiled by R.L.P. Adams together with four other authors at Glasgow University, and is a substantial revision of the last, 1976, edition. The book is published by Chapman & Hall and costs £8.50 in paperback.

of fossils, geographical position does not necessarily give us the correct taxonomic allocation of living organisms. Biological taxa should be recognized by biological criteria alone. Let the stratigraphical and biogeographical chips fall where they may.

According to Hall, about 400 species and subspecies have been added to the living North American mammalian fauna since 1959, bringing the present total up to 3,607. However, although myriads of named (and presumably approved) subspecies are listed and referenced, morphological evidence for their recognition is not presented. Rather, maps depict arbitrary boundaries between localities that produce "marginal specimens" of the putative subspecies. The characterizing features of these - if genuinely biological, and not merely geographically arbitrary or a relic of overly split typological taxonomy — must be sought either in the original literature or elsewhere. Perhaps, in some long-distant future revision, the subject of intraspecific variation can be handled quantitatively. Hall has made a start, but the job is immense.

Finally, Hall's treatment of higher taxonomic categories should be taken with a grain of salt. This is because he deals with members of the living fauna as though they had no history: he does not differentiate between primitive features and phylogenetically significant shared-derived

ones. For example, he seems to be unaware that characters such as an inflected mandibular angle or epipubic bones are not merely metatherian hallmarks but characterize early eutherians as well. Nor does he record that jugal bones reach the glenoid fossa in paenungulates, not just in the Metatheria. Interested readers should also run through the list of mainly primitive features supposedly characterizing Polyprotodontia, Didelphidae or Insectivora (the latter stretched to include the conceptually indigestible macroscelidids). Hall's concept of the "Order Pinnipedia" and his separation of living sloths from megalonychid ground sloths by interlarding the Myrmecophagoidea into their midst will also raise palaeontological and cladistic eyebrows alike.

Hall's work will continue to be useful in spite of its conceptual shortcomings and his apparently stubborn refusal to modify adequately some sections of the first edition which many, even most, mammalogists found objectionable — those dealing with bears, *Dama* and *Lasiurus*, for example.

After all these grumblings, do I recommend the monograph? Yes, of course. After all, what better summary do we have?

Malcolm C. McKenna is in the Department of Vertebrate Paleontology at the American Museum of Natural History, New York.

# An abundance of beetles

M.E.G. Evans

The Biology of the Coleoptera. By R.A. Crowson. Pp.802. ISBN 0-12-196050-1. (Academic: 1981.) £58, \$139.50.

WE LIVE in the age of the obscure beetle. Flowers, fleas, birds and butterflies are all much more obvious organisms, but there are more species in the order Coleoptera than there are in the class Angiospermae, and even, perhaps, than in the whole plant kingdom. Since this enormous species diversity is based upon a common body plan, the variations on this theme should be of great interest to evolutionary biologists as well as specialist entomologists. So where are the hosts of coleopteran biologists?

One reason why biological studies of beetles lag far behind their taxonomy may be a simple failure to appreciate the scope of the subject. What has been lacking until now has been a general biological text which deals with the numerous but scattered studies of beetles. It is just this sort of book which Dr Crowson has written; it is based on well over 1,000 references, including many from non-

English language literature, most of which is recent. However, it is more than a mere compendium of references. In most cases, the information on a topic has been summarized, digested and assimilated into a more general subject area in each of the 20 chapters of text, ranging from morphology and genetics to ecology and evolution. The basic approach is both functional and phylogenetic, although an extended treatment of classification is deliberately omitted. Nevertheless, a family classification is appended, and taxonomic groups in the text are given code numbers which refer to the phylogenetic chart following Chapter 20.

Inevitably, there are some blemishes. Over 300 figures are included, most of which are simple, clear, line drawings, but some have been drastically reduced for printing whilst a few are not reduced enough. Not surprisingly, some topics are discussed in less depth than others, but in most cases these differences in treatment reflect our different levels of comprehension of the subject. Indeed, this has enabled the author to point to obvious gaps

in our knowledge of the Coleoptera over; the whole range of their biology, making one of the most valuable features of the book.

Taken together with its encyclopaedic coverage, these indications of our ignorance will enable *The Biology of the Coleoptera* to provide many potential researchers with a starting point, a basic literature survey and a summary of current knowledge. This is one of the most important books on beetles to be published this century, and it is the first modern text on their biology. I hope it will be bought by many entomologists, evolutionary biologists and science libraries.

M.E.G. Evans is Senior Lecturer in Zoology at the University of Manchester.

# **Puzzles of perception**

Oliver Braddick

Direct Perception. By Claire F. Michaels and Claudia Carello. Pp.200. ISBN 0-13-214791-2. (Prentice-Hall: 1981.) £13.50, \$24.30.

THE perceptual psychologist J.J. Gibson (1904-1979) wrote a series of books which crystallized a theoretical stance he called "ecological optics" and "direct perception". The books were widely read and many details from them became absorbed as standard textbook material, but it seemed for many years that Gibson's basic position was a lonely, almost ignored one, outside the mainstream of thinking on perception. It is still outside the mainstream, but in the years just before and since Gibson's death it has gathered such a vigorous, even strident, group of younger advocates that no one in the field has an excuse for being unaware of it. There has been a particularly zealous Gibsonian cell in and around the University of Connecticut, two of whose members have produced this short, clear, didactic book.

The view against which the authors argue is that the perceiving organism takes in at the sense organs a collection of "raw data" whose relation to objects in the external world is distant and often incomplete, and that the task of perceptual psychology is to discover the computations by which information about the external world is obtained or inferred from these data. The alternative they propose is that perception has to be considered as a function of a system consisting of organism and environment, that the ecologically relevant properties of the environment are present as higher-order invariants in the pattern of energy reaching the senses, and may be detected because the perceptual system "resonates" directly to these higher-order invariants, rather than

computing or inferring anything. Some other advocates of this position have presented it in a manner that appeared to relish shocking, mystifying or finessing more conventional views; here the argument is more orderly and well paced, with its progress charted in summaries of each chapter and at the end of the book. An appendix, "Discussion and Debate", which deals with some queries and criticisms of the argument in question-and-answer form, is very helpful in clarifying just what the authors' position is, although as a debate it is distinctly stage-managed.

It is a valid criticism of much work in sensory and perceptual psychology that it does not give enough thought to what perception is for, or to whether the stimulus variables that are manipulated in perceptual experiments bear any relation to the variables the perceiver needs to use. The opponent that the book sets up is therefore not a straw man, but he lacks flesh in a way that the Gibsonians blithely ignore. The information-processing tradition is centrally interested in problems of mechanism; not only (indeed, not enough) in what job perceptual systems do, but also in how they work. For instance, Michaels and Carello argue that the direct perception approach undercuts the two 'puzzles in binocular vision" which have been "an object of discussion for three millenia" - how can a pair of twodimensional retinal images yield a 3-D percept, and how can it be single? No awareness is indicated that the major binocular puzzle that has interested scientists in the last two decades is not of this kind but is "What kind of mechanism finds pairs of corresponding elements in the two eyes' images?". Now analysis of ecological optics might suggest that this question should be posed in a somewhat different form, but identification of the relevant invariants in the optic array does not abolish the question, how does the system work which "resonates" to them? It is difficult to see how such an answer can be cast except in terms of signals relayed by the sense organs and operations performed upon these signals.

For this reason, scientists working in the information-processing and psychophysical traditions are likely to be impatient with much of Direct Perception. What they would find it hard to deny is that most of the experimental work generated by the direct perception school has been interesting - something they would have to be very partisan to assert of their own tradition. It is perhaps a pity that Michaels and Carello did not make their book 50 pages longer and give us a more detailed awareness of how their approach bears scientific fruit - in the experimental and analytical work of Lee, Shaw and Turvey, among others.

Oliver Braddick is a Lecturer in Experimental. Psychology at the University of Cambridge.

# **Bones** into stones

Mark Newcomer

Life History of a Fossil: An Introduction to Taphonomy and Paleoecology. By Pat Shipman. Pp.222. ISBN 0-674-53085-3. (Harvard University Press: 1981.) \$25, £14.

PALAEONTOLOGISTS are no longer content to classify, weigh, measure and tabulate fossil bones, and are bringing their subject out of the museum through studies of the processes by which the fossil record forms (taphonomy) and through reconstructions of extinct animal communities in their environmental setting (palaeoecology). Recent work in these fields, which to some degree parallels that of archaeologists who attempt to see their sites and artifacts in their geographical context, is the subject of Pat Shipman's lively text.

Much of the book concentrates on the principles, methods of study and results of taphonomic studies of (mainly) African vertebrates, reflecting the author's research interests and competence. She treats her material critically, steering a cautious course through such murky waters as the spiral fracture debates, and stresses the value of suitable quantitative methods for many taphonomic and problems; palaeoecological comments on excavation procedures and "living floors", however, seem a little naive, especially in light of all the recent publications on these matters. (Why does the long reference list contain only Englishlanguage publications? Curious in a book intended as a text.) Without sounding arrogant, I think it is fair to say that standards of excavation have generally been higher in archaeology than in palaeontology, with the fossil hominid hunters perhaps the most culpable, relying on the spectacular nature of their finds to obscure excavation methods which are often mediocre or worse. Similarly, the author could have gone more deeply into the role of hominids in creating both the types and spatial patterns of refuse on sites, given recent experimental work and ethnographic studies by Binford and Yellen, among others.

Intended for "advanced undergraduate courses in anthropology, palaeontology, and evolutionary biology", as well as professionals in these fields, *Life History of a Fossil* will serve as an excellent introduction to taphonomy and palaeoecology. It should also reach a much wider audience with less specialized interests in vertebrate evolution, to whom the lessons to be learned from humble, often broken, bones will come as a pleasant surprise.

Mark Newcomer is a Lecturer in Palaeolithic Archaeology at the Institute of Archaeology, London.

# OKS RECEI

#### **Mathematics**

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CHURCHOUSE, R.F. (ed.). Handbook of Applicable Mathematics. Vol.III, Numerical Methods. Pp.565. ISBN 0-471-27947-1. (Wiley: 1981.) £32.50, \$75.

COX, D.R. and SNELL, E.J. Applied Statistics Principles and Examples. Pp.189. Hbk ISBN 0-412-16560-0; pbk ISBN 0-412-16570-8. (Chapman & Hall: 1981.) Hbk £13.50; pbk £6.95.

MILLER, R.G. Jr. Simultaneous Statistical Inference, 2nd Edn. Pp.299. ISBN 3-540-90548-0. (Springer-Verlag: 1981.) DM 44, \$21.

#### **Physics**

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BRUNDLE, C.R. and BAKER, A.D. Electron Spectroscopy: Theory, Techniques and Applications, Vol.4. Pp.500. ISBN 0-12-137803-9. (Academic:

BUTTON, K.J. and WILTSE, J.C. (eds). Infrared and Millimeter Waves. Vol.4, Millimeter Systems. Pp. 364. ISBN 0-12-147704-5. (Academic: 1981.) \$46. EMRICH, R.J. (ed.). Fluid Dynamics Part A. Methods of Experimental Physics,

Vol.18. Pp.403. ISBN 0-12-475960-2. (Academic: 1981.) \$50.

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0-12-283860-2. (Academic: 1981.) £27.60, \$66.50. GRAY, K.E. (ed.). Nonequilibrium Superconductivity, Phonons, and Kapitza Boundaries. Based on the Proceedings of a NATO Advanced Study Institute held August 1980 in Acquafredda di Maratea, Italy. NATO Advanced Study Institutes, Series B. Physics. Pp.699. ISBN 0-306-40720-5. (Plenum: 1981.) \$85.

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HULL, H.H. An Approach to Rheology through Multivariable Thermodynamics or Inside the Thermodynamic Black Box. Pp.158. Hbk ISBN 0-9606118-0-9; pbk ISBN 0-9606118-0-0. (Harry H. Hull, c/o Deeds Associates, Pittsburgh: 1981.) Hbk \$24; pbk \$16.

SEIPPEL, R.G. Optoelectronics. Pp.354. ISBN 0-8359-5255-X. (Prentice-Hall: 1981.) £16.45.

SWINNEY, H.L. and GOLLUB, J.P. (eds). Hydrodynamic Instabilities and the Transition to Turbulence. Topics in Applied Physics, Vol.45. Pp.292. ISBN 3-540-10390-2 (Springer-Verlag: 1981.) DM 96, \$50.40.

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WILKINSON, D. Progress in Particle and Nuclear Physics, Vol.7. Pp.315. ISBN 0-08-027152-9. (Pergamon: 1981.) £36.50, \$84.

#### **Technology**

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JONES, O.C. Jr. (ed.). Nuclear Reactor Safety Heat Transfer. Proceedings of the International Centre for Heat and Mass Transfer, 12. Pp.959. ISBN 0-89116-224-0. (Hemisphere Publishing: 1981.) \$99.

NORMAN, A. and LITTLE, A.D. Electronic Document Delivery. The ARTEMIS Concept for Document Digitalisation and Teletransmission. A Study Prepared for the Directorate-General Information Market and Innovation. Commission of the European Communities. Pp.233. Pbk ISBN 0-904933-29-6. (Learned Information (Europe), London: 1981.) Pbk £29. SOMERSCALES, E.F.C. and KNUDSEN, J.G. (eds). Fouling of Heat Transfer

Equipment. Proceedings of an International Conference held at Rensselaer Polytechnic Institute, August 1979. Pp.743. ISBN 0-89116-199-6. (Hemisphere Publishing: 1981.) \$75.

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DE SAINT-MAUR, G.P. (chief ed.) Geology of Continental Margins (C<sub>3</sub>).

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LINELL, K.A. and TEDROW, J.C.F. Soil and Permafrost Surveys in the Arctic. Pp.279. ISBN 0-19-857557-2. (Clarendon Press/Oxford University Press: 1981.)

PATERSON, W.S.B. The Physics of Glaciers. 2nd Edn. Pp.380. Hbk ISBN 0-08-024005-4; flexi ISBN 0-08-024004-6. (Pergamon: 1981.) Hbk £20, \$48, Can. \$60; flexi £7.25, \$17.50, Can. \$28.

REYMENT, R.A. and BENGTSON, P. (eds). Aspects of Mid-Cretaceous Regional Geology. Pp.328. ISBN 0-12-587040-X. (Academic: 1981.) £30, \$72. SMITH, A.G., HURLEY, A.M. and BRIDEN, J.C. Phanerozoic Paleocontinental World Maps. Pp.102. Hbk ISBN 0-521-23257-0; pbk ISBN 0-521-23258-9. (Cambridge University Press: 1981.) Hbk £15; pbk £6.95

SPOSITO, G. The Thermodynamics of Soil Solutions. Pp.223. ISBN 0-19-857568-8. (Clarendon Press/Oxford University Press: 1981.) £24.

SWANSON, E.A., STRONG, D.F. and THURLOW, J.G. (eds). The Buchans Orebodies. Fifty Years of Geology and Mining. The Geological Association of Canada, Special Paper 22. Pp.350 + maps. ISBN 0-19216-18-8. (Geological Association of Canada, University of Waterloo, Canada: 1981.) Np.

WHITTAKER, E.J.W. Crystallography. An Introduction to Earth Science (and Other Solid State) Students. Pp.266. Hbk ISBN 0-08-023805-X; flexi ISBN 0-08-023804-1. (Pergamon: 1981.) Hbk £13.50, \$32.50; flexi £8.35, \$19.95.

### **Biological Sciences**

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AKERT, K. (ed.). Biological Order and Organization. Selected Works of W.R. Hess. Pp.347. ISBN 3-540-10551-4. (Springer-Verlag: 1981.) DM 128, \$67.20.

ALBERT, M.L. et al. Clinical Aspects of Dysphasia. Disorders of Human Communication, 2. Pp.194. ISBN 3-211-81617-8. (Springer: 1981.) \$422, DM 59. ALEXANDER, R.D. and TINKLE, D.W. (eds). Natural Selection and Social

Behavior: Recent Research and New Theory. Pp.532. ISBN 0-913462-08-X. (Blackwell Scientific/Chiron: 1981.) £35. ALEXANDER, R.M. The Chordates. 2nd End. Pp.510. Hbk ISBN

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AUSTIN, C.R. and EDWARDS, R.G. (eds). Mechanisms of Sex Differentiation

in Animals and Man. Pp.604. ISBN 0-12-068540-X (Academic: 1981.) £37, \$89. BAYLIFF, W.H. (ed.). Inter-American Tropical Tuna Commission. Special Report No.2, Synopses of Biological Data on Eight Species of Scombrids. Pp.530. (no ISBN) (Inter-American Tropical Tuna Commission, La Jolla, California: 1980.) Pbk np.

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BIGNON, J. and SCARPA, G.L. (eds). Biochemistry, Pathology and Genetics of Pulmonary Emphysema. Proceedings of an International Symposium held in Sassari, Italy, April 1980. Pp.428. ISBN 0-08-027379-3. (Pergamon: 1981.) £27.

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Vol.72. Pp.329. ISBN 0-12-364472-0. (Academic: 1981.) \$38.

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ISBN 0-12-147608-1. (Academic: 1981.) \$47.50.
BUTTERWORTH, T. and LADDA, R.L. Clinical Dermatology, Vol.2. Pp.355. ISBN 0-03-059139-2. (Holt, Rinehart & Winston: 1981.) Np.

CALOW, P. Invertebrate Biology: A Functional Approach. Pp. 183. Hbk ISBN 0-7099-0000-7; pbk ISBN 0-7099-0001-5. (Croom Helm, London: 1981.) Hbk £11.95; pbk £5.95.

# ANNOUNCEMENTS

#### **Awards**

UC-San Francisco neurologist **Dr Robert** Layzer, has been presented with the 18th annual J. Elliott Royer Award for his contribution to psychiatry and neurology.

The National Association of Science Writers has presented its Science-in-Society Journalism awards for 1981. The winners are: Newspapers — Paul Jacobs, science reporter, the Los Angeles Times, for his series of articles entitled: "Pioneer Genetic Implants". Broadcast News — Ira Flatow, science editor, NPR News, for his two-part broadcast on All Things Considered "Creationism vs. Evolution". Magazines — Janet Raloff, science reporter, Science News, for "Electromagnetic Pulse;" shared with William J. Broad Jr, Science for "Nuclear Pulse."

The Chemical Writers' Awards, sponsored by the Society of Chemical Industry and BASF UK Ltd is this year open to radio and television material as well as the written word. The premier award is the Chemical Writer of the Year, the other classes are: Chemical Reporter of the Year; chemical Application Writer of the Year; and Agrochemical Writer of the year. Material must have been published and circulated or broadcast, in the UK between October 1980 and 31 December 1981. Entries close on 31 January 1982. Entry forms from: Wynn Jones, BASF UK Ltd, PO Box 4, Earl Road, Cheadle Hulme, Cheadle, Cheshire, UK.

Entries are invited for the 1982 Achievement Award presented by the Worshipful Company of Scientific Instrument Makers. The award is intended to bring added prestige to the British scientific instrument industry, to draw attention to new and original products, and to highlight British progress in the field of scientific instruments and electronic technology. Details of suitable achievements should be submitted to the Clerk to the Company, Scientific Instrument Makers Hall, 9 Montague Close, London SE1, UK by the end of April 1982.

The British Vacuum Council seeks to encourage scientists under 27 years of age by inviting entries for its Annual C.R. Burch Prize of £150 for the best submitted paper on vacuum studies, surface science, thin films, or any related topic in which vacuum science or engineering has an important role. Entries by 31 December 1982 to Dr J.S. Collington, Dept of Electrical Engineering, University of Salford, Salford, UK.

## Meetings

21-23 December, Trigger Processes and Cellular Events, London (Miss A. Duffey, Dept of Biochemistry, Royal Free Hospital, School of Medicine, 8 Hunten St, London WC1, UK).

3-8 January, 1982 AAAS National Meeting, Washington DC (A. Herschman, AAAS, 1776 Massachusetts Ave, NW, Washington DC 20036, USA).

5 January, Higher Education: Demand and Access, London (Dr I. Gibbs, The College, Lord Mayor's Walk, York, UK). 5-7 January, Compaction of Particulate Solids, Bradford (Institution of Chemical Engineers, 12 Gayfere St, London SW1, UK).

7 January, Electron Microscopy of Cement and Concrete, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

11 January, **Dosimetry in Diagnostic Radiology**, London (Dr. D.W. Field, Hospital Physicists' Association, 47 Belgrave Square, London SW1, UK).

12 January, Evolution from Space, London (Omni Magazine, 2 Bramber Rd, London W14, UK).

15 January, Long-range Surveillance of Ocean Surface Wind and Sea State by Ground-based Radar, Edinburgh (Royal Meteorological Society, James Glaisher House, Grenville Place, Bracknell, Berks, UK).

19-21 January, Membrane Recycling, London (Director, The Ciba Foundation, 41 Portland Place, London W1, UK).

20 January, Planetary Meteorology, London (Royal Meteorological Society, James Glaisher House, Bracknell, Berks, IJK)

21 January, Sources of Geological Information for the Caribbean and Latin America, London (Joan Hardy, Library, Chelsea College, Manresa Rd, London SW3, UK).

25 January, Immunochemistry Today, London (School Office, Royal Postgraduate Medical School, Du Cane Rd, London W12, UK).

25-30 January, **Technical Conference on Climate in Africa**, Arusha (World Climate Programme Office, World Meteorological Organization, Geneva, Switzerland).

27 January, Solar Energy for Developing Countries, London (The Administrator, UK-ISES, 19 Albemarle St, London W1, UK).

28 January, The Use of Icebergs, Cardiff (Royal Meteorological Society, James Glaisher House, Grenville Place, Bracknell, Berks, UK).

28-29 January, Maintanance of Deionisation and Reverse Osmosis Plant, High Wycombe (The School of Water Sciences, The Lorch Foundation, Lane End, High Wycombe, Bucks, UK).

\*18-22 January, Electrochemistry, Ventura

\*1-5 February, Chemistry and Biology of Peptides, Ventura

\*1-5 February, Sensory Transduction in Microorganisms, Ventura

\*8-12 February, Cardiovascular Role of Brain Angiotensin and other Peptide Hormones, Ventura

\*8-12 February, **Metal in Biology**, Ventura \*15-19 February, **Alcohol**, Ventura

\*Gordon Research Conferences, Dr A.M. Cruickshank, Pastore Chemical Laboratory, University of Rhode Island, Kinston, Rhode Island 02881, USA.

8-11 February, Protein Structure and Function, Lorne (Dr R.J. Simpson, St Vincent's School of Medical Research, Victoria Parade, Fitzroy, Victoria, Australia).

8-12 February, International Society for Human and Animal Mycology Congress, Palmertston North (The Secretariat, PO Box 63, Palmerston North, New Zealand).
8-12 February, Reticuloendothelial Society Congress, Davos (Prof. E. Sorkin, Schweizerisches Forschuninstitut, Medizinische Abtelling, 7270 Davos-Platz, Switzerland).

15-19 February, Occupational Health and Safety and the Law, Loughborough (The Centre for Extension Studies, Loughborough University of Technology, Loughborough, Leics., UK).

15-22 February, International College of Surgeons Congress, New Dehli (Dr Atm Prakash, C-1/12 Medical Enclave, New Dehli, India).

18 February, Advances in Cardiovascular Imaging, London (British Institute of Radiology, 36 Portland Place, London W1, UK).

19 February, **The Future in Radiology**, London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

25-26 February, Representing Understanding, London (Dr E.W. Shepherd, Dept of Psychiatry, Guy's Hospital Medical School, London Bridge, London SE1, UK).

26-27 February, **Physiological Society Meeting**, Bristol (Dr D.C. Michel, University Laboratory of Physiology, Parks Rd, Oxford, UK).

26-27 February, **Paediatric Oncology**, London (Royal College of Radiologists, 38 Portland Place, London W1, UK).

28 February — 5 March, Scanning Electron Microscopy in the Biological Sciences, Woods Hole (M.D. Maser, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA).

8-10 March, WMO/EPPO Symposium on Meteorology and Plant Protection, Geneva (WMO Secretariat, Research and Applications Programme Dept, Geneva, Switzerland).

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QUEEN Elizabeth Hospital — Birmingham Research Technician (Temporary) required in Dept of Medicine for 1 yr only. Research project concerning the isolation and characterization of immune complexes in human glomerulonephritis. Salary £4,220, or on scale £4,958 to £6,993 a for State Registered MLSO's. Applicants with good Hons degree in Biological Sciences, preferably with some grounding in immunology, should submit a full CV to Personnel Officer, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, from whom further information is available. Closing date: 24th December 1981. (034)A

#### THE MOUNT WILSON AND LAS CAMPANAS OBSERVATORIES of the CARNEGIE INSTITUTION

# OF WASHINGTON Announce the availability of a STAFF MEMBERSHIP

The Observatories seek to make an appointment to their scientific staff in 1982. Staff Members have access to the C1W telescopes on Mount Wilson, on Palomar Mountain, and on Cerro Las Campanas, Chile. A cooperative arrangement between the Carnegie Institution and the California Institute of Technology permits access to the facilities of Caltech's Palomar Observatory.

There is no restriction with regard of field of inquiry, but the person appointed will have at least three years' experience beyond the PhD and will have an established record of achievement in astronomical esearch. Those who might like to be considered for the appointment are nvited to write a letter expressing heir interest to:

Dr. George W. Preston, Director, Mount Wilson and Las Campanas Observatories, 813 Santa Barbara Street, Pasadena, CA 91101-1292, J.S.A.

The letter should be accompanied by a brief curriculum vitae, sibliographic reference to three of he applicant's published scientific apers, and the names and addresses of three scientists familiar with the pplicant's research.

The closing date for the receipt of hese letters will be January 15, 1982.

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#### UNIVERSITY OF HAMBURG (Germany)

# TECHNICIAN FOR DNA (GENE) SYNTHESIS

Technicians who like to join a research group dealing with DNA synthesis and recombinant DNA research are requested to apply for this position. Experiences in nucleic acid synthesis is of advantage but not necessary. The position is at least for 2 years.

Salary is about DM 25,000 brutto. Applications, together with curriculum vitae, and the names of two referees should be sent to: Prof Dr H Köster, Institut für Organische Chemie and Biochemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, Federal Republic of Germany. (W529)A

#### Astronomical Image Processing MOUNT WILSON AND LAS CAMPANAS OBSERVATORIES

# CARNEGIE INSTITUTION OF WASHINGTON

The Observatories are seeking a person with experience in image processing to design, assemble, and upgrade software packages for a VAX 750 computer, located at the Observatories' headquaters in Pasadena, California. The primary responsibility will be de develop useroriented systems for the analysis of digitized photographic plates and two-dimensional data from CCD detectors. Additionally, there may be opportunities for collaborative scientific research with the staff and fellows of the Observatories.

Applicants should have an educational background in either astronomy or computer science, with substantial experience in image processing. The position could be suitable for a scientist seeking postdoctoral experience or for a career data analyst.

Interested persons should send résumés to:

Dr. Alan Dressler, Mount Wilson and Las Campanas Observatories, 813 Santa Barbara Street, Pasadena, CA 91101-1292, U.S.A.

The closing date for the receipt of applications for this position is January 15, 1982.

The Carnegie Institution of Washington is an Equal Opportunity Employer, M/F/H. (NW140)A

# UNIVERSITY OF SOUTHERN CALIFORNIA

DEPARTMENT OF BIOCHEMISTRY AND COMPREHENSIVE CANCER CENTRE

#### ASSISTANT PROFESSOR

Molecular biologist experienced in recombinant DNA techniques and their application to study eukaryotic differentiation. Department of Biochemistry and Comprehensive Cancer Centre, University of Southern California (USC), An Equal Opportunity Employer.

Send CV, bibliography, four references, and a statement of present and future research interests to: Dr Charles Heidelberger, USC Cancer Center, 1721 Griffin Avenue, Los Angeles, CA 90031.

(NW149)A

# UNIVERSITY OF SASKATCHEWAN

DEPARTMENT OF BIOLOGY

Postdoctoral position available to investigate the liposome-mediated transfer of chromosomes into auxotrophic cells of *Datura*.

Candidates should have a strong background in genetics and cell biology and be trained in the use of plant cell culture techniques.

The position is available immediately for a one-year term (renewable for a further two years) at a salary of \$16.380 Can.

Candidates should send curriculum vitae and two letters of recommendation to Dr John King, Department of Biology, University of Saskatchewan, Saskatoon, Sask, Canada S7N OWO. (NW153)A

# nature

has an immediate vacancy in London for an

# ASSISTANT EDITOR

to work primarily on the assessment of physical science manuscripts.

Essential qualifications:

- \* A good science degree
- Some knowledge of the earth sciences
- Some research experience
- A sense of literacy
- \* Wide general interests.

Applications, including a full curriculum vitae, should be plainly marked "Job application" and addressed to the Editor, Nature, Macmillan Journals, 4 Little Essex Street, London WC2R 3LF to arrive before 15 December 1981. Secondment for not less than two years would be appropriate.

(9927)A

# Chief Executive Officer

The Saskatchewan Research Council is seeking a Chief Executive Officer to lead the organization into expanded fields of research in natural resources, energy, industrial engineering and the environment.

The Saskatchewan Research Council, a provincial Crown agency, is one of Canada's major institutions devoted to technological development. Its staff of about 250 undertakes an extensive program of internally initiated research and experimental development, as well as performing contractual and fee-for-service work on behalf of industry and government. Located on the University of Saskatchewan campus in Saskatoon, the Council also has a SLOWPOKE research reactor, new chemical analytical laboratories and pilot plant facilities situated in the adjacent research park.

Saskatchewan is a rapidly-growing and diversifying province. The economy, based on agricultural, mining and energy industries, is forecast to sustain one of the highest rates of growth in Canada during the decade. Saskatoon is a stimulating education, research and business centre noted for its quality of life. Offering a high level of cultural activities, extensive recreational facilities, and affordable homes, the city maintains a friendly community environment.

The Chief Executive Officer will work with leaders in business, government, research and the community at large to foster the role of science and technology in the province. The new Chief Executive Officer will chart the future course for the Saskatchewan Research Council and, with proven skills, will see those concepts and plans implemented. With an established scientific or engineering background, the Chief Executive Officer will be a pragmatic individual committed to Saskatchewan's technological development.

Please reply, in confidence, to the Chairman, Chief Executive Officer Search Committee:

Dr. Douglas Patriquin Executive Council Government of Saskatchewan 3085 Albert Street REGINA, Saskatchewan Canada, S4S 0B1

(NW154)A

# Saskatchewan Research Council

# CSIRO AUSTRALIA

# Research Fellow (Molecular Biology)

\$A19,662 — \$A28,564 pa Molecular & Cellular Biology Unit North Ryde, New South Wales

**CSIRO** has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,500 employees — 2,700 of whom are research and professional scientists — located in divisions and sections throughout Australia.

**General:** The Unit's work includes research programmes on animal cell growth and development, DNA maintenance and modification and gene technology.

**Duties:** To research into DNA repair and mutagenesis and mechanisms of action of bleomycins and phleomycins.

Qualifications: A PhD, or equivalent. Basic training in biochemistry microbiology or microbial genetics.

Tenure: Three years. Superannuation benefits available.

Applications: In writing and stating full personal and professional details, the names of at least two referees and quoting reference No A3314 should reach: The Officer-in-Charge, Molecular & Cellular Biology Unit, CSIRO, PO Box 184, NORTH RYDE, NSW 2113, AUSTRALIA by 8 January 1982. (048)A

## WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales)

SOUTH WALES
RADIOTHERAPY AND

ONCOLOGY SERVICE Velindre Hospital, Whitchurch, Cardiff CF4 7XL

# RESEARCH OFFICER IMMUNOLOGY

A vacancy exists for a suitably qualified graduate to investigate biophysical aspects of white cell interactions with specific and nonspecific stimulants. A wide range of equipment is available to study various aspects of cellular response and an innovative approach will be required to develop a new research programme in association with existing flurorescent techniques. A physical or biological background is desirable since the emphasis will be on a physical approach to biologically orientated phenomena.

The post will last for an initial period of three years with salary dependent upon qualifications but in the range £5,285 to £7,700 per annum.

Further details may be obtained from Dr J A V Pritchard, Head, Immunology Department, Velindre Hospital, Whitchurch, Cardiff CF4 7XL, Tel: 615888.

Application forms (quoting ref No R30) obtainable from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN. Tel. No. 0222/755944 ext 2296. (030)A

# UNIVERSITY OF THE WEST INDIES

Jamaica

Applications are invited for two post of

# PROFESSOR/SENIOR LECTURER IN PHYSICS

One appointment will be made in the field of Materials Science. The appointee is expected to continue to shape and teach undergraduate as well as (projected) graduate courses; the person appointed will also be expected to lead in the further development of research in the area of Materials Science. Industrial experience will be an advantage.

The other appointment should be in one of the following fields: Atmospheric Physics; Radio Astronomy; Energy Studies; Materials Science (Metals and Fibres). The appointee is expected to consolidate and/or develop the research interests of the Department, especially the one(s) which coincide with his/her own interests. Salary scales: Professor J\$22,848 — J\$28,284 pa. Senior Lecturer J\$17,748 — J\$21,717 pa. (£1 sterling = J\$3.37). FSSU. Study and Travel Grant. Unfurnished accommodation or housing allowance. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica.

Applicants resident in UK should also send 1 copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 10 Spring Gardens, London SW1A 2BN. Further details are available from either address. (022)A

#### THE OPEN UNIVERSITY



# **Faculty of Science BIOLOGY ELECTRON** MICROSCOPE **TECHNICIAN**

The Science Faculty has a tenured vacancy in the Biology Discipline for an experienced Technician to maintain and operate the department's Electron Microscope facilities The unit consists of a Joel JEM 100S and a Philips 301 with ancillary equipment housed in a purpose built suite including a photographic darkroom. Thin sectioning equipment includes a Reichert OMU3 and a Reichert ultracut.

Access to scanning electron microscope facilities is also available.

This post offers an opportunity to a technician seeking a responsible position in a unit serving both the Teaching and Research needs of the Department.

Applicants should have an HNC or equivalent and 10-12 years' practical experience in Biological Electron Micrscopy.

Commencing salary will be within the Technician Grade VI scale £6,532 — £7,802 p.a.

Application forms and further particulars are available from the Assistant Secretary, Science (438/2), The Open University, Walton Hall, Milton Keynes, MK7 6AA or telephone Milton Keynes (0908) 653481: there is a 24 hour answering service on 653868.

Closing date for applications: 31st December.

### **UNIVERSITY OF** SOUTHAMPTON DEPARTMENT OF PHYSICS POST DOCTORAL RESEARCH **FELLOW**

Applications are invited from Physicists with interests in experimental high energy particle physics. The work involves the development and use of hardware for such experiments and also on-line and off-line computing which forms an important part of this activity. The Fellow will be joining a group which is involved in experimental work at CERN (Geneva) and will be expected to spend part of his/her time there. Starting salary will be in the range £6,070 — £7,700 per annum plus USS benefits

Applications (2 COPIES) should be sent to R A Dawson, Staffing Department, The University, Highfield, Southampton SO9 5NH with curriculum vitae and the names of referees quoting reference 2001/R/N. (045)A



# Research & Development diagnostic reagents

# Scientific and technical appointments

**BECKENHAM: KENT** 

Wellcome Reagents has an established place in the diagnostics market. The Reagents Research and Development team has a wide ranging brief, from the initial research and feasibility studies to final product development, and enjoys close liaison with specialist marketing and production colleagues. Collaboration with other scientific groups, participation in symposia and publications are important as part of the work of the department. As a result of continuing expansion we can offer challenging opportunities at both scientist and technician level.

# SCIENTIFIC APPOINTMENTS

Applications are invited from graduate and post doctoral scientists with at least 2-3 years working experience in either of the following areas:

## IMMUNOASSAY METHODOLOGY

with particular emphasis on radio immunoassay or related non isotopic techniques. A preference for the development and evaluation of methods, rather than simple experience of routine use, would be an advantage.

# CLINICAL BACTERIOLOGY

knowledge and experience in the field of medical microbiology and associated bacterial immunology is essential. Suitable candidates are likely to have spent part of their career in a

# TECHNICAL APPOINTMENTS

Suitable candidates will have some years' relevant laboratory experience of biochemical, immunochemical or immunoassay techniques. Formal education to HNC level is expected and opportunities will be available for further study where appropriate.

Salaries for all positions will reflect the experience and abilities of appointees. Excellent benefits include 5 weeks' holiday, subsidised restaurant, first class sports and social facilities and generous assistance with relocation expenses where appropriate.

For an application form please ring our automatic telephone answering service on 01-650 6541, giving your name and address and quoting reference number WRL/159. Alternatively please write to Miss A. Rountree, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3



# POSTDOCTORAL POSITIONS

Funded for 2 years are available immediately to study phospholipid metabolism in the cardiovascular system. The research will focus on the isolation, characterization, and regulation of cardiovascular phospholipases A2.

Salary: NIH guidelines.

Send résumé and three letters of reference to: Dr Richard C Franson, Department of Biophysics, Box 694, Medical College of Virginia, Richmond, Va. 23298.

An Equal Opportunity/Affirmative (NW147)A Action Employer.

The Faculty of Science of the University of Geneva is looking for candidates to fill a position of

# **PROFESSOR**

of general and/or cellular physiology, for the department of Animal Biology.

The successful candidate is expected to participate in teaching of general phisiology for biology students and to lead a strong research

Application: To be sent before 15 March, 1982 to: Faculté des Sciences, Université de Genève, 20, Quai Ernest Ansermet, CH-1211 Genève 4, Switzerland.

# Program Director, Immunology

Play a key role in advancing the field of *in-vitro* diagnostics.

The BBL Microbiology Systems Division of Becton Dickinson is the world leader in microbiology. Combine your scientific expertise with our recent state-of-the-art advances, and together we'll assume the same leadership position in the expanding area of in-vitro diagnostics.

You'll provide overall direction to our expanding Immunology department by managing existing projects and by assessing needs for new ones. Using a "systems approach" to management, you'll develop and monitor budgets, secure necessary external resources, develop detailed plans and schedules, etc.

You should possess a PhD or equivalent and formal training in immunology, virology, biochemistry, microbiology and/or cell biology. You should have working knowledge of the growth and isolation of microbial antigens, production of conventional and monoclonal antibodies, and biochemical and immunodiagnostic technologies. Scientific/industrial recognition as evidenced by publications, patents, etc., is required, as is a minimum of 8 years research experience including a supervisory background.

We offer an excellent salary and outstanding benefits. Find out more about the innovative environment and the career advantages of this expanding division of Becton Dickinson by sending résumé and salary history to: Dr. Laurance McCarthy, BBL Microbiology Systems, Becton Dickinson, Dept. 20D PO Box 243, Cockeysville, Maryland 21030. An equal opportunity/affirmative action employer.

Caring for people is our business

BECTON DICKINSON

(NW138)A

# UNIVERSITY OF THE WEST INDIES

Jamaica

Applications are invited for the following posts

#### 1 CHAIR IN COMPUTING SCIENCE

in the Department of Mathematics. Applicants will be expected to have wide interests and experience in Computing Science and to have done considerable research in Software Development and/or Applications.

2 GUINNESS RESEARCH FELLOW/JUNIOR RESEARCH FELLOW IN GEOLOGY

(Peat Studies) tenable for two years

Primary consideration will be given to persons who have a postgraduate qualification in a field related to peat science, particularly in palynology or palaeontology. Those with a suitable first degree will be considered at the Junior level. The appointee will be required to carry out stratigraphical and palaeontological investigations on the late Quaternary deposits of coastal wetlands in Jamaica. He/she will also expected to do a certain amount of lecturing and/or seminar work in related Quaternary geological topics to advanced undergraduate and postgraduate students, and to participate in setting up the research facilities necessary for the continuation of work in this field.

Salary Scales: Professor J\$22,848 — J\$28,284 pa. Research Fellow J\$15,090 — J\$20,049 pa. Junior Research Fellow J\$13,308 — J\$14,088 pa. (£1 sterling = J\$3.37). FSSU. Study and Travel Grant. Unfurnished accommodation or housing allowance. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Applicants resident in UK should also send 1 copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 10 Spring Gardens, London SW1A 2BN. Further details are available from either address.

(023)A

### UNIVERSITY OF TEXAS

DEPARTMENT OF MICROBIOLOGY POSTDOCTORAL POSITIONS

Virologist, immunologist and experimental oncologist. Part of an active research program on interferons, lymphokines, cytokines, effector cells and anti-tumor agents.

Send C V and names and telephone numbers of three references to:

Dr Samuel Baron, Department of Microbiology, The University of Texas Medical Branch, Galveston, Texas 77550

An Equal Opportunity/ Affirmative Action Employer, M/F/H. (NW151)A

# THE LONDON HOSPITAL MEDICAL COLLEGE (University of London) Electron Microscope Unit DEPARTMENT OF ORAL PATHOLOGY SENIOR MLSO IN ELECTRON MICROSCOPY

A vacancy exists for an experienced microscopist in this busy and forward-looking unit. The unit is involved in a variety of research projects and has the most modern equipment for both transmission and scanning electron microscopy. Applicants should have full experience of preparative procedures for either or both techniques.

Salary will be at a point on the Whitley Council Senior Medical Laboratory Scientific Officer scale and applicants should have FIMLS, MI Biol or an equivalent qualification. Informal enquiries and appointments to visit the unit may be made by telephoning on 01-247 5454, ext 348.

Formal applications, quoting names of two referees, within fourteen days, to Professor N W Johnson, Department of Oral Pathology, The London Hospital Dental Institute, Turner Street, London El 2AD. (036)A

CELLULAR PHYSIOLOGIST urgently needed. Teaching and research experience emphasized. For partipation in current work on cell culture of transformed cells. Please apply to the Chairman, Physiology Department, Ponce School of Medicine, P.O. Box 7004, Ponce, PR 00732 U.S.A. (NW150)A

# THE INSTITUTE FOR ADVANCED STUDY

will have several openings for members in theoretical physics and astrophysics for the academic year 1982-83. The positions are at a post-doctoral or higher level and applicants will be selected on the basis of their ability to do research in the areas of elementary particles, mathematical physics, astro-physics, plasma physics, general relativity and statistical mechanics. Preference is given to candidates who have received their PhD within the last year or two.

Postdoctoral members frequently collaborate with each other, with faculty members at the Institute or Princeton University, and with researchers at other institutions.

Appointments are usually for no more than two years and support is typically full salary for postdoctorals and half salary for more senior persons. Women and minorities are encouraged to apply.

For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW1006)A

## CCD Detector Development MOUNT WILSON AND LAS CAMPANAS OBSERVATORIES

# CARNEGIE INSTITUTION OF WASHINGTON

The Observatories are seeking a person with experience in detector development to participate in the construction of multiple-CCD imaging systems for large-area, multi-color photometric measurements. The detector systems will be assembled at the Observatories' headquaters in Pasadena, California, and installed on Carnegie telescopes at Palomar Mountain, California and Cerro Las Campanas, Chile. Participants in the development effort will have the opportunity to engage in research programs using the completed instruments.

Applicants should have an educational background in either astronomy, physics, or electronics, with some experience in detector development and computer data acquisition. The position could be suitable for a scientist seeking postdoctoral experience or for an instrumentation professional.

Interested persons should send résumés to:

Dr. Stephen A. Shectman, Mount Wilson and Las Campanas Observatories, 813 Santa Barbara Street, Pasadena, CA 91101-1292, U.S.A.

The closing date for the receipt of applications for this position is January 15, 1982.

The Carnegie Institution of Washington is an Equal Opportunity Employer, M/F/H. (NW139)A

# UNIVERSITY OF LEICESTER DEPARTMENT OF GENETICS POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from persons with a good background in biochemistry or molecular genetics for a postdoctoral research position financed by a Cancer Research Campaign award. The project will be concerned with the characterisation of error-prone DNA replication in *E. coli* induced by carcinogens or UV-irradiation. This will involve the use of cloning teachniques, in vitro DNA replication systems and enzyme purification procedures. The work is likely to involve close collaboration with research groups in the Department studying other aspects of the SOS response in *E. coli* and with groups studying the control of and mechanism of plasmid DNA systhesis using an in vitro system.

The appointment will be tenable for up to 3 years from 1 January 1982 or as soon after as can be arranged. Starting salary £6,880 (at age 26) per annum with full superannuation provision.

Applications with names of two referees to Dr I B Holland, Department of Genetics, University of Leicester, Leicester LE1 7RH.

(043)A

# **ORGANIC CHEMISTS**

# -for Medical Products Development £8500-£9000

We need skilled and imaginative Organic Chemists to play key roles in our development programme for new medical products.

Our diagnostic kits are already well established in the Obstetrics, Thyroid and Haematology fields and make a substantial and growing contribution to our turnover worldwide. The opportunities for further growth are considerable and these posts require innovative Organic Chemists who, as members of multi-disciplinary development teams, can take responsibility for the design and preparation of novel compounds for incorporation into proposed clinical assay kits.

Candidates must be qualified to PhD level in Organic Chemistry or have equivalent experience. The posts offer considerable scope for scientists keen to join a rapidly expanding, high technology organisation.

Starting salaries will be around £8500 - £9000. Conditions of employment are excellent and assistance with relocation will be given where appropriate.

In the first instance, please write with full career details to P. L. Jones, Personnel Manager, at the address below:

# **Amersham International Limited**

White Lion Road Amersham Buckinghamshire HP7 9LL

(052) A



# MEDICAL RESEARCH COUNCIL DIVISION OF VIROLOGY NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Applications are invited for a short term staff appointment with tenure for 3-5 years to join a small team engaged in morbillivirus research. The work currently involves exploiting recombinant DNA techniques and the successful applicant will be expected to develop this approach. Experience in biochemical, virological and/or recombinant DNA technology at a post-doctoral level would be desirable.

Salary will be dependent on age and experience and will fall within the range £7290-£8925 plus £967 London allowance and superannuation provision. Further information may be obtained from Dr W C Russell (01-959 3666 Ext 273).

Applications should be submitted to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA by the 1st February 1982 together with Curriculum vitae and the names of two professional referees. Please quote reference V1/LH. (040)A

# Senior Research Microbiologist

Cutter Laboratories, Inc., the world leader in plasma fractionation, and manufacturer of a broad range of medical and biological products for international health-care markets, seeks a Senior Research Microbiologist to head a research team to investigate the use of monoclonal antibodies for therapy. The research facilities are housed in a brand-new multimillion dollar building.

This position requires the knowledge ordinarily acquired from post-doctoral research in Microbiology and Immunology or an equivalent combination of education and experience. The successful candidate must have demonstrated ability to carry out independent and innovative research in the field of monoclonal antibodies, and possess a good knowledge of cellular immunity.

Cutter Laboratories is located in Berkeley, California, which is part of the very cosmopolitan San Francisco Bay Area. Within this area are also located the Berkeley and San Francisco campuses of the University of California.

Cutter Laboratories offers excellent salaries, competitive with comparable positions in this area, along with attractive fringe benefits.

Please send detailed resume to Corporate Employment Department, PO Box 1986, Berkeley, CA 94701. An equal opportunity/affirmative action employer.



CUTTER Laboratories, Inc.

(NW148)A



# THE SPACE SCIENCE DEPARTMENT

of the

## **EUROPEAN SPACE AGENCY**

seeks

#### **SCIENTISTS**

for

## **EXOSAT OPERATIONS**

Applications are invited from young scientists holding postdoctoral or equivalent qualifications in astronomy or physics and having experience with scientific instrumentation and data analysis (preferably related to space research) to act as duty scientists in the operation of the European X-Ray Observatory Satellite EXOSAT.

Duties include evaluation of the feasibility of observation proposals, establishment of observation programmes, liaison with observers, conduct of observations and preliminary data analysis. The scientists will have the opportunity of undertaking their own observation programmes in X-Ray astronomy.

Prior to launch the scientists will be located at ESA's Space Science Department ESTEC in the Netherlands, transferring to ESOC, Darmstadt in Germany for orbital operations.

Please send detailed curriculum vitae to the Head of Personnel ESTEC, Postbus 299, 2200 AG Noordwijk, the Netherlands.

(W532)A

# UNIVERSITY OF GIESSEN Faculty of Biology

# **HEAD OF MICROBIOLOGY**

Applications from German speaking individuals are invited for the position of a full PROFESSORSHIP (C4). The successful applicant is expected to direct teaching of biology students in microbiology and should have demonstrated research excellence in the area of gene expression and gene regulation including experience in recombinant DNA methodology.

Applications should be received before 5th of January 1982, and should include curriculum vitae, list of publications, account of teaching experience and the names of three referees. The position will become available in the summer of 1982. Applications should be addressed to:

President, University Glessen, Postfach 111 440, D-6399 Glessen, Federal Republic of Germany

(W525)A

# Scientists for Condensed Matter Studies at Rutherford Appleton Laboratory

The Rutherford Appleton Laboratory is constructing a new neutron source, known as the Spallation Neutron Source (SNS) which will provide intense pulsed neutron beams for condensed matter research by scientists from universities and polytechnics. This is a major project of world significance which is planned for initial operation by 1984.

Preparation for exploitation of the SNS has begun, and requires progressive recruitment of neutron scattering scientists to support the neutron scattering experimental programme, and theoretical scientists to work with the associated condensed matter theory group led by Dr S W Lovesey. Neutron scattering specialists will contribute to the design of instruments, supervision of their operation, planning of support facilities, and collaboration with external university scientists. The work of the theory group is presently directed to the static and dynamic properties of localised magnets, electron motion and correlations in metals and semiconductors, liquid crystals and phase transitions, and the dynamic properties of simple liquids and macromolecular systems. Staff are encouraged to participate in scientific research projects, usually in collaboration with the user community, and using facilities at Harwell and the Institute Laue – Langevin (ILL) Grenoble.

At present the Laboratory is able to offer two permanent posts at SO/HSO level. There is also scope for fixed term appointments and joint appointments with universities. Applications are invited from suitable qualified scientists, both experimental and theoretical, who have already demonstrated their ability in condensed matter research.

Salary scales:

Scientific Officer £5176-£6964 Higher Scientific Officer £6530-£8589

For further details and an application form, contact the Personnel Group on Abingdon (0235) 21900 quoting reference VN02 or write to the Personnel Group, Rutherford Appleton Laboratory, Chilton, Didcot, Oxon. OX11 0QX.

Closing date for applications: 6th January, 1982.

(055)A

Rutherford	
Appleton	In the forefront
Laboratory	of research

# UNIVERSITY OF EDINBURGH DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH FELLOW (Crystallography)

Applications are invited for the above SRC-funded post to investigate the crystal structures of some simple volatile inorganic compounds.

Applicants should have experience in X-Ray Crystallography. Familiarity with techniques of low temperature crystal preparation would be an advantage.

The appointment will be for two years starting on or after 1st April 1982. The initial placing will be on the 1A scale, starting at a point that will depend on age and qualifications.

Applications, including the names of two referees, should be sent to Professor E A V Ebsworth or Dr A J Welch, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, from whom future particulars may be obtained. Please quote reference 5049. (029)A

# UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY

Applications are invited from candidates with a good honours degree in Biology, Microbiology or Public Health Engineering for a post of

## RESEARCH ASSISTANT

in the above Department for a fixed period of three years commencing not later than 1st March 1982. The successful candidate will work on an SERC funded research project on the treatment of domestic sewage in waste stabilization ponds in warm European climates and will be expected to do up to two months fieldwork each year in Lisbon, Portugal.

Initial salary within the range £5,285 — £6,070 per annum.

Applications, together with the names of three referees, should be received not later than 5th January, 1982, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/912/N. (025)A

# UNIVERSITY OF THE WITWATERSRAND

Johannesburg

DEPARTMENT OF PHYSIOLOGY

Applications are invited from suitably qualified persons, regardless of sex, religion, race, colour or national origin, for appointment to the posts of: SENIOR

## LECTURER/LECTURER

Applicants for these posts should have a medical or veterinary degree or a PhD in Physiology, Biochemistry, Zoology or any other biological field.

The Lectureships and Senior Lectureships involve teaching undergraduate and graduate Medical and Science students, research, and participation in the administrative work of the department. There is no restriction on the field of interest of applicants.

The salary range for Lecturer is R10,995 — R19,230 and for Senior Lecturer is R14,370 — R20,850 per annum

Intending applicants are requested to obtain copies of the information sheet relating to these vacancies from the Director, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE (quoting ref 11/1/698), or from the Director: Personnel Office, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001, South Africa, with whom applications should be lodged by 31 December 1981, or as soon thereafter as possible.

(032)A

# Professor of Mechanical Engineering Royal Military College of Science

Applications are invited for the permanent post of Professor and Head of the Department of Mechanical Engineering. The Department, with 24 teaching and 70 supporting staff, is responsible for the CNAA BSc courses in Military Vehicle Technology, Guided Weapons, and Gun Systems, Design. There are branches in Applied Thermodynamics, Applied Mechanics and Mechanical Design, each headed by an Associate Professor.

The College runs first degree, post-graduate, army staff and special courses on a wide variety of subjects. While its primary function is the scientific education of army officers, the student population includes civilians, and military personnel from overseas. It is a residential establishment occupying a large estate in the Vale of the White Horse, near Swindon, and has an academic staff of over a hundred. There are

extensive laboratories and workshops; and research is encouraged, and supported by a variety of outside sponsors. The role of the college is being reviewed. The successful applicant will be required to take part in implementing any changes that are decided by the Army Board.

Applicants should be Mechanical Engineers of distinction and achievement, preferably with experience of teaching at university level.

Starting salary (according to qualifications and experience) within the range £17,685 – £20,895.

For further details and an application form (to be returned by 8 January 1982) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref:* S/5673/5. (038)A

# UNIVERSITY OF CAPE TOWN ASSOCIATE PROFESSOR OR SENIOR LECTURER IN GENETICS

Applications are invited for the above post in the Department of Biochemistry. Appointment, according to qualifications and experience, will be made on the salary scale:

Senior Lecturer: R14,370  $\times$  810-R20,850 pa. Associate Professor: R18,420  $\times$  810-R20,850  $\times$  900-R22,650 pa. In addition a service bonus of nearly one month's salary is payable annually.

Candidates should have strong research interest in eucaryotic molecular genetics and experience in recombinant DNA technology and assume duties during the first half of 1982. Candidates should state for which post they wish to be considered.

The post may also be filled at the request of the applicant on a contract basis for 2-3 years with either the possibility of renewal or conversion into a tenured post.

Applicants should submit curriculum vitae, stating present salary, research interest and publications, when available if appointed, and the names and addresses of three referees.

Further information should be obtained from The Registrar (Attention Appointments Officer) University of Cape Town, Rondebosch, 7700 South Africa to whom applications (Quoting reference No AC/11) must be submitted as soon as possible.

The University's policy is not to discriminate in the appointment of staff on the grounds of sex, race or religion. Further information ont he implementation of this policy is obtainable from the Registrar. (W518)A



# UNIVERSITY OF BIRMINGHAM



# **DEPARTMENT OF PHYSICS**

The Department of Physics has a limited number of places for suitably highly qualified overseas students wishing to undertake postgraduate work to MSc or PhD level. Candidates should be in the top 15% of their degree class and have, or expect to get, the equivalent of a top honours degree. Selected candidates will be nominated for the U.K. Government Fee Support awards.

The Physics fields of interest include

# CONDENSED MATTER NUCLEAR STRUCTURE ELEMENTARY PARTICLES and APPLIED NUCLEAR SCIENCE

For full details of the research activities and related MSc courses, and for application forms please write to

Dr. Malcolm C. Scott Department of Physics University of Birmingham P.O. Box 363 Birmingham B15 2TT, England.

(027)A

#### UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts:

#### LECTURESHIP/SENIOR LECTURESHIP **DEPARTMENT OF LAND MANAGEMENT** (Available 1st March, 1982)

Applicants must have a degree in Soil Science with major qualifications in either Soil Chemistry or Soil Physics. Experience in teaching and research would be an advantage.

### LECTURERSHIP/SENIOR LECTURESHIP **DEPARTMENT OF CLINICAL PHARMACOLOGY** (2 posts) (available immediately)

Applicants must have a medical qualification with a specialist diploma such as MRCP or equivalent. The successful applicant will be required to teach Pharmacology and Clinical Pharmacology to Medical and Pharmacy students, and to assist in the management of General Medical wards.

#### LECTURESHIP/SENIOR LECTURESHIP DEPARTMENT OF MEDICAL MICROBIOLOGY (Available 1st March, 1982)

The successful applicant should preferably have experience in all fields of medical microbiology but an interest in virology would be an advantage. Duties would include lecturing to Medical Pharmacy and para-medical students and also to supervise the day-to-day running of the diagnostic virology unit situated in the Department.

#### LECTURESHIP/SENIOR LECTURESHIP **DIVISION OF BIOLOGICAL SCIENCES** (BIOMATHEMATICS) (Available 1st March, 1982)

A Biomathematician required with experience in the teaching of statistical and analytical methods as well as in the mathematical modelling of natural systems to each courses at undergraduate and postgraduate levels and to advise on the design and analysis of research carried out by students and staff in the Division of Biological Sciences.

#### SCIENTIFIC DIRECTOR UNIVERSITY LAKE KARIBA RESEARCH STATION **DIVISION OF BIOLOGICAL SCIENCES** (Available 1st March, 1982)

Applicants should be scientists with an established record of leadership and personal research achievement in the study of aquatic ecosystems to lead a team of mainly post-doctoral research fellows based at Kariba which will investigate the Lake Kariba system, the probable initial emphasis of this research being on primary production and nutrient

## LECTURESHIP/SENIOR LECTURESHIP **DEPARTMENT OF BOTANY** (Available immediately)

The successful applicant, who must have a suitable doctorate, will be expected to contribute to courses in general and applied microbiology at both undergraduate and postgraduate degree level. Preference will be given to candidates with research experience in applied or environmental bacteriology.

Salary Scales (approx. Stg. equivs.):

Non-Medical — Academic Staff Lecturer Grade II: £5,046 × 363 — £6,861 × 380 — £8,761 Lecturer Grade II: £9,158 × 380 — £10,679 Senior Lecturer: £10,109 × 380 — £11,249 × 389 — £13,194

Medical - Academic Staff

Medical — Academic Starr
Assistant Lecturer:£7,551 × 311 — £8,795
Lecturer Grade II: £9,910 × 371 — £12,139
Lecturer Grade II: £12,519 × 380 — £14,040
Senior Lecturer: £14,472 × 432 — £16,200 × 363 — £16,563

Appointment on the above scales according to qualifications and experience.

Conditions of Appointment: Both permanent pensionable terms and short-term contracts are offered for academic posts.

Further Particulars on conditions of service and on method of application should be obtained prior to submitting an application from application should be obtained plant to director, Appointments and Personnel, University of Zimbabwe, PO Roy MP 167 Mount Pleasant, Salisbury, Zimbabwe, or the Box MP, 167, Mount Pleasant, Salisbury, Zimbab Association of Commonwealth Universities (Appts.), Square, London WC1H 0PF.

Closing date for receipt of applications is 31 January 1982.

(049)A

# I.L.R.A.D. (Nairobi)

Applications are invited from suitably qualified candidates for the post of:

# POST DOCTORAL FELLOW Ref No PDF/L4/81/1

A position is available for a post-doctoral fellow to work with the Immunobiology Unit on the definition of cells of the bovine immune system. Applicants must have experience in preparing, maintaining and testing, functional T-cell, natural killer cell etc., clones from a mammal.

The successful applicant will be expected to work closely with other members of the unit who have produced monoclonal antibodies against bovine B-cell T-cell and macrophage surface membrane determinants. The studies are directed towards understanding bovine immune response against African trypanosomes and Theileria infected bovine cells. This is an international position, the salary will be paid in U.S.\$ and the successful applicant will be assisted with moving expenses

Applications identified with Ref No including C.V., summary of PhD thesis, and three references should be sent to:

> Chief Personnel Officer, I.L.R.A.D P.O. Box 30709, NAIROBI, Kenya

Closing date: 7/1/82

(W528) A

#### UNIVERSITY OF ARIZONA DEPARTMENT OF CHEMISTRY

ACADEMIC POSITION

Invites applications for a possible tenure-track position at the Assistant Professor level in one of the following areas: (1) Synthetic Solid State Chemistry; (2) Surface Chemistry; and (3) Atmospheric or Low-Temperature Geochemistry.

Candidates should have demonstrated in their Ph D and/or postdoctoral work the ability to develop a vigorous and innovative research program in one or more of the above areas and have a commitment to instructional excellence.

résumé brief description of research plans, and three letters of recommendation should be sent to Professor William S Glaunsinger, Chairman, Search Committee, Department of Chemistry, Arizona State University, 85287. EO/AA employer. (NW146)A State University, Tempe, Arizona

#### UNIVERSITY OF OXFORD DEPARTMENT OF ZOOLOGY DEPARTMENTAL **DEMONSTRATORS**

Applications are invited for two demonstratorships, the holders of which will be required to assist in the teaching work of the department, particularly the Animal Kingdom course. One post will be primarily concerned with the invertebrate part of the course and this will be available for 1st October 1982. The other is concerned with the vertebrate course and should be taken up in January 1982 or as soon as possible afterwards. The posts are tenable for years and may be renewed for one further period; the salary scale is £8,105 £5.285 -

Candidates, who should be of post-doctoral level, should send their applications with the names of two referees and a clear indication of their preference and availability for the wo posts to the Linacre Professor, Department of Zoology, South Parks Road, Oxford by 5th January.

(046)A

# UNIVERSITY OF CAPE TOWN

**Department of Organic Chemistry** 

# Postdoctoral or Post MSc **Research Fellowship**

A Fellowship is available at either the postdoctoral or post MSc level for research in organic chemical synthesis, with emphasis on aromatic, quinonoid, and heterocyclic systems of potential medicinal interest. The position will be available for one year in the first instance, and renewable for a further two years.

Salary ranges: Postdoctoral R10 935 - 13 410 Post MSc R8 730 - R11 430

A contribution to relocation expenses may be made for overseas appointees. Further information may be obtained from Associate Professor R.G.F. Giles, Department of Organic Chemistry, University of Cape Town, Rondebosch, 7700, South Africa, to whom applications, with the names of two referees should be sent as soon as possible.

# **EXCHANGE CAPITAL FOR IDEAS**

# IN BIOLOGICAL OR CHEMICAL COMPOUNDS

We are not a pharmaceutical firm but a Swiss-based, independent financial group which is prepared to let you participate in a capital venture for the exploitation of your project.

In 1981, we have examined more than 400 projects.

Our object: the development of new biological or chemical products with previous pharmacological screening up to the stage of clinical tolerance and efficacy required to permit licensing negotiations.

To obtain all the information required for initial selection, please write in English, French or German to: DEBIOPHARM S.A. Petit-Chêne 38 - 1001 LAUSANNE (Switzerland).

(W527)A

#### CARLETON UNIVERSITY DEPARTMENT OF GEOLOGY

# Invites applications for the position of RESEARCH ASSOCIATE

with responsibility for the electron microprobe laboratory.

Candidates should have appropriate background to work with the department's Cambridge MKV mciroprobe, Ortec EDS, Data General Nova 2 computer, PESTRIP-4 and EDDATA data handling programs and the University's Honeywell CP-6 computer. This is a two-year position with the possibility of renewal.

Send curriculum vitae and the names of three referees before January 15, 1982 to J A Donaldson, Chairman, Department of Geology, Carleton University, Ottawa, Ontario K1S 5B6 Canada.

(NW144)A

# UNIVERSITY COLLEGE DEPARTMENT OF PATHOLOGY POST DOCTORAL

# RESEARCH POSITION

Post doctoral research position under the National Board for Science and Technology "Higher Education Industry Cooperation" scheme commencing January-February 1982. Project entitled "Pharmaceutical applications of monoclonal antibodies". Experience of tissue culture techniques essential.

Salary will be commensurate with qualifications and experience up to IR£7,500. Project is funded for 3 years.

Send Curriculum Vitae with the names and addresses of two referees to Dr H McLaughlin, Dept of Pathology, University College, Earlsfort Tce, Dublin 2, to arrive before December 21st 1981.

(037)A

# ROSENSTIEL SCHOOL OF MARINE AND ATMOSPHERIC SCIENCE

# University of Miami

Tenure track position in Fisheries Science in Division of Biology and Living Resources: Assistant Professor to Professor; salary and rank dependent on qualifications and experience; starting date 1 June 1982 or as soon as possible thereafter.

or as soon as possible thereafter.

Applicants should hold Ph.D. in fisheries or appropriate related field. Experience desirable in one or more of following areas: a) stock assessment and management; b) tropical, multi-species fisheries; c) larval fish ecology; d) predator-prey relationships. Incumbent expected to develop field and laboratory research including independent research proposals and would be responsible for teaching graduate courses "Introduction to Fisheries Science" and "Biometrics" and development of new courses in area of specialization as required. The candidate would be encouraged to initiate or participate in fisheries projects overseas.

Applications should include curriculum vitae, a brief statement of research interests and objectives and names of three referees and be forwarded to:

Dr. F. Williams, Chairman, Search Committee, Division of Biology and Living Resources, Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, Florida 33149.

Closing date for applications 19 February 1982.

The University of Miami is an Affirmative Action/Equal Opportunity Employer. (NW143)A

# CSIRO AUSTRALIA Research Fellow (Cellular Biology)

\$A19,662 — \$A28,564 pa Molecular & Cellular Biology Unit North Ryde, New South Wales

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,500 employees — 2,700 of whom are research and professional scientists — located in divisions and sections throughout Australia.

**General:** The Unit's work includes research programs on animal cell growth and development, DNA maintenance and modification and gene technology.

**Duties:** As a member of the growth and development group, to carry out research on Cellular Differentiation, particularly on the mechanisms of action of specific differentiation factors.

Qualifications: A PhD, or equivalent, in biological sciences, with basic training in some aspects of molecular biology. Experience with tissue culture techniques is essential.

Tenure: Four years, superannuation benefits available.

Applications: In writing and stating full personal and professional details, the names of at least two referees and quoting reference No A0506 should reach: The Officer-in-Charge, Molecular & Cellular Biology Unit, CSIRO, PO Box 184, NORTH RYDE, NSW 2113, AUSTRALIA by 8 January 1982. (047)A



## Rijksuniversiteit Utrecht

State University of Utrecht, Netherlands

The Department of Geology and Geophysics of the State University of Utrecht, The Netherlands invites applications for the position of

# **Professor in Meteorology**

Applicants will be responsible for the teaching of meteorology. The candidate will also be expected to participate in and encourage research, and to contribute to national and international coordination in the field of meteorology.

Preference will be given to atmosphere scientists or physicists with a scientific qualification and interest in geophysical fluid dynamics.

Candidates should be willing to learn to lecture in Dutch within two years. Salary is to a maximum of Dfl. 10.844, — per month, depending on age and experience.

Applications, including a curriculim vitae and list of publications, should be directed, within 4 weeks upon publication of this journal, to the application board, Instituut voor Meteorologie en Oceanografie, Princetonplein 5, 3584 CC Utrecht, Netherlands.

Further information can be provided there. Tel: 030-533275. (W530)A

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# Scientific Specialists I Microbiology I Immuno-diagnostics Our client is the Diagnostics Division of one of the world's leading health care companies, with an outstanding range of diagnostic products in the forefront of technology.

- forefront of technology.
- These two opportunities are based at European headquarters in Wiesbaden, Germany, and provide technical services, R & D activity and scientific advice within an international marketing group. Considerable travel throughout Europe and elsewhere will be involved.
- Candidates should have a PhD degree or equivalent, with appropriate laboratory experience either in microbiology or clinical chemistry/immuno diagnostics depending on the appointment. A multi-lingual capability in English and French or German essential. A good scientific background with a commercial awareness is required.
- Salaries and benefits are very attractive. Opportunities for further promotion are genuinely interesting.

Send a cv to J F Fulford, Grosvenor Stewart, 207-209 Avenue Louise, 5th Floor, Box 8, 1050 Brussels; or 117 George Street, London W.1; or telephone Belgium (02) 6400796 or UK (0462) 55303.



# GROSVENOR STEWART

International Management Recruitment

(031)

**UNIVERSITY OF EDINBURGH** DEPARTMENT OF **HUMAN GENETICS** 

#### RESEARCH ASSOCIATE/ **POST-DOCTORAL FELLOW**

Applications are invited for the above post, funded by the cystic fibrosis research trust and tenable from 1st March 1982 for 18 months in the first instance. Candidates should be capable of establishing and maintaining a laboratory for culture of human fibroblasts. Experience in hybridoma technology would be an advantage.

Starting salary at an appropriate point according to qualifications, age and experience on either scale 1A for post-doctoral fellows (£6070-10575) or scale 1B (£5285-7700). Curriculum vitae should be sent as soon as possible to Dr D J H Brock, Depart-ment of Human Genetics, Western General Hospital, Edinburgh 4., from whom further particulars can be obtained (031-332 2471). Please quote reference 5069. (042)A

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S **HOSPITAL** 

(University of London) West Smithfield, London EC1A 7BE

#### POST DOCTORAL RESEARCH FELLOW

required in Department of Haematology for Cancer Research Campaign grant entitled "Isolation and Characterisation of regulatory variants for the expression of murine lymphocyte cell surface antigens". Experience in cell membrane biochemistry necessary and immunology/cell culture experience desirable. The post is tenable for three years from 1 January 1982, or soon after. Salary on Scale £6,070 — £10,575 plus £967 LA.

Apply in writing with CV and names of two referees to The Secretary of the College at the above address, quoting ref 925. Further information from Dr M A Horton Tel: 01-600 9000 exten-(021)Asion 3272.

Please mention

# nature

when replying to these advertisements

IMPERIAL COLLEGE University of London **POSTDOCTORAL** RESEARCH ASSISTANT IN

## PHYSICAL CHEMISTRY

Required to develop electro-chemical sensors for real time trace element analysis of seawaters. A background in electroanalytical chemistry and micro-computer techniques would be advantageous. The appointment is for one year in the first instance. Salary up to £7,874 (inc L/A). Start as soon as possible.

Apply with cv and names of two referees to Professor W J Albery, Department of Chemistry, Imperial College, London SW7 2AY.

(050)A

#### ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

#### RESEARCH ASSISTANT

required to work on the biochemistry of human cardiac valves. The project will involve techniques in protein separation and cell culture and applicants should have a good honours degree in Biochemistry or a nonours degree in Biochemistry or a related discipline. The appointment is funded by the Jenson Research Foundation, available from 1 March 1982 and for 1 year initially renewable for up to 3 years. Initial salary up to £7044 p.a. There may be an opportunity to register for a higher degree with the University degree with the University.

particulars Further application form available from the Establishments Officer, St George's Hospital Medical School, Cranmer Terrace, London SW170RE.

#### UNIVERSITY OF OXFORD DEPARTMENT OF GEOLOGY AND MINERALOGY POST-DOCTORAL

RESEARCH ASSISTANT A Research Assistantship is available for three years, from 1 January 1982. for a geochemist with a strong background in physical chemistry or a physical chemist with interests in geology to assist in the measurement of the thermodynamic properties of silicate melts and minerals by high temperature Knudsen Cell mass spectrometry. The research is in col-laboration with the Max-Planck-Institut für Chemie in Mainz, W Germany and will involve approximately three months work per year in Germany.

Salary £6880 - £7700 plus living expenses in Germany.

Applicants should Curriculum Vitae and two letters of recommendation to Dr D G Fraser, Department of Geology and Mineralogy, University of Oxford, Parks Road, Oxford, OX1 3PR.

(041)A

# UNIVERSITY OF NORTH CAROLINA DEPARTMENT OF BIOCHEMISTRY

#### RESEARCH ASSISTANT **PROFESSOR** PhD contingent upon funds,

develop the research and training program in regulation of expression of genes involved in DNA repair, particularly to study the genes for the enzymes involved and their control. Postdoctoral training required.

Send CV and 3 letters of reference to: Nancy Nye, Dept of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, NC 27514.

We are keenly interested in attractwe are keenly interested in attracting outstanding women and/or minority scientists to this department. EQUAL OPPORT-UNITY/AFFIRMATIVE ACTION EMPLOYER. (NW145)A

#### **UNIVERSITY OF** WISCONSIN

DEPARTMENT OF METEOROLOGY

#### POSTDOCTORAL PROJECT **ASSOCIATE**

Position available to participate in observational and theoretical studies of Arctic stratus clouds and related problems in Arctic meteorology.

Research includes analysis and interpretation of meteorological and radiometric data from aircraft field programs, and related modelling studies and parameterization development. 1-3 years duration. Start approximately 1 February 1982.

Salary commensurate with experience and qualifications. Ph D in meteorology, physics or related fields. Send vitae and names of three references to GF Herman, Meteorology, University of Wisconsin, Madison, WI 53706 by 20 January 1982. An Equal Opportunity (NW152)A Employer.

#### SIMON FRASER UNIVERSITY

Burnaby, British Columbia, Canada

DEPARTMENT OF BIOLOGICAL **SCIENCES** 

#### **ENVIRONMENTAL** TOXICOLOGY

Applications are invited for two tenure-track positions, one at the Assistant Professor level and one at the Associate Professor level. The successful applicants will teach undergraduate courses in En-vironmental Toxicology and graduate courses in Industrial Toxicology and Food and Drug Toxicology. Candidates must have a PhD degree with experience in toxicology and a strong record in research. The successful applicants will be expected to develop and maintain an active research programme in an area of toxicology appropriate to a Life Sciences department. Preference will be given to candidates eligible for employment in Canada at the time of application.

The positions are available from 1 September 1982 subject to budgetary constraints. The current salary base for the Assistant and Associate Professor ranks are \$25,000 and \$31,600/yr respectively.

Applications should include a curriculum vitae, a brief statement of research interests and objectives, and selected reprints of published research. Applicants should request confidential assessments of their research and teaching ability from three referees, to be forwarded directly to: Dr K K Nair, Chairman, Dept of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6.

Dealine for receipt of applications is 31 January 1982, or until the positions are filled. (NW129)A

### CONFERENCES and **COURSES**

#### UNIVERSITY OF BRISTOL

12 MONTHS M Sc COURSES BY ADVANCED STUDY AND RESEARCH are offered in the following

subjects:
Advanced Analytical Chemistry (Inorganic Chemistry) Surface Chemistry and Colloids (Physical Chemistry) The Physics of Materials (Physics) Mathematical Logic and Foundations of Mathematics (Mathematics) Fluid Mechanics (Mathematics)

Meat Science (School of Veterinary Science, Langford)

All six courses qualify for SERC studentships. Early application is fesirable. Further details of the ndividual courses may be obtained rom the Head of Department concerned, University of Bristol BS8 TH. Enquiries are also invited for letails of opportunities for research eading to the degree of PhD.

**UNIVERSITY OF SURREY** DEPARTMENT OF BIOCHEMISTRY **MSc IN TOXICOLOGY** 

Applications are invited for a postgraduate course leading to an MSc degree in toxicology. The course, which may be attended either fulltime or part-time is organised by the Department of Biochemistry, University of Surrey, with the MRC Toxicology Unit, Carshalton; the British Industrial Biological Research Association, Carshalton and Shell Toxicology (Tunstall) Laboratory, Sittingbourne. During the course instruction is given in these collaborative laboratories as well as at other major toxicological institutions. Entrance requirements are as for a PhD and a limited number of grants from the Medical Research Council and the Inveresk Research Foundation are available for suitable candidates. On the successful completion of the course opportunities may be available for students to register for a PhD in toxicology, and to undertake a period of research training in one of the above named organisations or in other laboratories approved by the University as being suitable for toxicology research.

The Medical Research Council, the Royal Society and the Royal College of Pathology have identified toxicology as a priority area for research training and therefore support the encouragement of first class students from a variety of disciplines to become involved in the science of toxicology. In modern society contact with potentially toxic chemicals is increasing. Problems may arise from the use of drugs for medical purposes. from occupational exposure to industrial products or intermediates and from environmental exposure to natural or

synthetic substances.

The course, which was the first of its kind in Europe, is designed to provide graduates in science, and medical and veterinary graduates, with an understanding and appreciation of the many disciplines involved in toxicology. Particular emphasis is placed on aspects related to the biochemical mechanisms of chemically-induced toxicity, and the assessment of toxicological hazard in the manufacture, use and disposal of new chemical compounds, pesticides and medicines. The course provides a broad biological education and is a suitable introduction for those intending to undertake research on the interaction of chemicals with biological systems.

There are numerous interesting opportunities for scientists, educated initially in different disciplines, who have acquired additional knowledge of toxicology. These may be in industry, government regulatory bodies, or research institutes with good career prospects in both laboratory work and administration.

Further information and application forms to be returned by either 1st March, 1982, (British and EEC applicants) or 1st June 1982 (Overseas applicants), can be obtained from: Dr R S Jones, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH. (026)C

# **GULBENKIAN INSTITUTE OF** SCIENCE, PORTUGAL

## **During 1982 the following advanced** courses will be offered:

Electron Transfer Proteins, 21 June-2 July, J. Le Gall (Univ. Georgia), A.V. Xavier, J.J.G. Moura (New Univ. Lisbon), Larry C. Sieker (Univ. Washington).

The Biology of Human Social Relations, 19 July-30 July, Lionel Tiger, Robin Fox (Rutgers Univ.), James Chisholm (Univ. New

Effects of Alcohols and Other Membrane-Active Drugs on Yeast and Other Microorganism, 30 August-10 September, Anthony H. Rose (Univ. Bath), L.O. Ingram (Univ. Florida), Vitor M.C. Madeira (Univ. Coimbra), N. van Uden (Gulbenkian Inst.

Comparative Historical Sociology, 20 September 1 October, Richard M. Smith (Cambridge Group Hist, Pop. & Soc. Structure), Robert Rowland (Gulbenkian Inst. Sci.).

Taxonomy and Identification of Yeasts, 20 September-15 October, N.J.W. Kreger-van Rij (Univ. Groningen), C.P. Kurtzman (NRRC, Peoria, III.), J. Fell (Univ. Miami), S.A. Meyer (Georgia State Univ.).

Somatic Cell Genetics, 25 October-5 November, C. Thomas Caskey, David Konecki (Baylor Coll. Med., Houston), István Raskó (Biol. Res. Center, Szeged, Hungary).

Information and Registration Forms from: Professor N. van Uden, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal.

#### **LECTURES**

# NATO ADVANCED STUDY INSTITUTE

# The Application of Laser Light Scattering to the Study of Biological Motion

20 June - 3 July 1982, Maratea, Italy

Speakers: N.S. Allen, R.D. Allen (Dartmouth), J.P. Boon (Bruxelles), F.D. Carlson (John Hopkins), B. Chu (Stony Brook), H.Z. Cummins (CCNY), V Degiorgio (Pavia), J.C. Earnshaw (Belfast), R.P.C. Johnston (Aberdeen), D.E. Koppel (Connecticut), D.B. Sellen (Leeds), M.W. Steer (Belfast), B. Ware (Syracuse), K.E. Wohlfarth-Bottermann (Bonn), H Yu (Wisconsin).

Scientific Programme

The aim of the meeting is to bring together physical scientists and biologists to explore the use of laser light scattering methods in biological research. Lectures, which will be comprehensible to members of the 'alternate discipline', will cover the following areas:

- Introduction: physical principles and biological problems
  Techniques: laser Doppler microscopy, fluorescence techniques, data interpretation
- In vitro studies: diffusion, electrophoresis, gels, model membranes, vesicles, muscles
- Whole cell movement: bacterial and sperm motility, amoeboid
- Intra-cellular motion: cytoplasmic streaming, vesicle migration, molecular motion in cytoplasm

Further details and registration forms are available on request. Completed application forms must be submitted by 28 February 1982 to Dr. J. C. Earnshaw, Department of Physics, The Queen's University, Belfast, N Ireland BT7 1NN. (028)K

# **STUDENTSHIPS**

## THE HANNAH RESEARCH INSTITUTE Post-graduate Research Studentship

Applications are invited for a 3-year studentship sponsored by an industrial company to carry out research into the heat induced gelation reactions of milks. The successful applicant will carry out research in the Chemistry Department of the Hannah Research Institute in Ayr and will be registered for a higher degree in the Department of Dairy Science of the University of Glasgow.

Applicants should have a first or upper second class honours degree in Chemistry or other suitable discipline.

Applications, including the names of two academic referees. should be sent by 11th January to:-

The Secretary, The Hannah Research Institute, AYR, Scotland KA65HL

### **FELLOWSHIPS**

# **EMBO**

**European Molecular Biology Organisation** 

# LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1982 AWARDS

Next deadline: February 19, 1982

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but subject to review of progress they are usually renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The followship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is February 19, 1982. Successful candidates will be notified of the their awards immediately after the meeting of the selection committee which is on April 30, 1982.

Application forms and further details may be obtained from Dr J Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1,F.R. Germany.
(W510)E

# POST-DOCTORAL FELLOWSHIP

(Mechanisms of bacterial mutagenesis and DNA repair)

Applications are invited from persons currently working in an EEC country other than the UK, or non-UK EEC citizens currently working outside the Community. Previous experience in this or a related field is essential.

Write to Professor B A Bridges, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton, England. (024)E

#### THE UNIVERSITY OF AUCKLAND New Zealand REMOTE SENSING RESEARCH UNIT

A Postdoctoral Fellowship is available in the Remote Sensing Research Unit for a period of 12 months commencing during 1982. Applicants must have completed a PhD or its equivalent before being eligible to take up the Fellowship. Expertise in digital processing techniques and remote sensing applications is desirable. The unit's current research interests include practical resource analyses of NASA and NOAA satellite data, analysis of seasurface temperatures and mapping of land resources. A monthly allowance of NZ\$1,519 will be paid.

Further information including application forms are available from Dr G R Cochrane, Department of Geography, University of Auckland, Private Bag, Auckland, New Zealand. Applications close 30 August 1982. (051)E

# UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF CIVIL ENGINEERING

#### RIDLEY FELLOWSHIP IN AIR AND WATER POLLUTION

Applications are invited, from persons who have shown themselves able to carry out original research, for the Ridley Fellowship in Air and Water Pollution tenable in the Public Health Engineering Division of the Department of Civil Engineering. The Fellowship is financed by the British Steel Corporation to promote research in some aspect of air and water pollution which is of interest to the steel industry. The proposed topic is biochemical transformations in the biological treatment of coke oven liquors but consideration will be given to other research topics proposed by the applicant.

The normal tenure of the Fellowship will be three years, tenable from 1 April 1982 or a date to be arranged. The value of the Fellowship is £6,070 (first year), £6,475 (second year) and £6,880 (third year).

Further particulars and application forms (which must be returned by 31 January 1982) may be obtained from the Registrar (Fellowships), University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE17RU. (044)E

CANCER SOCIETY of New Zealand

#### BRUCE CAIN MEMORIAL POSTDOCTORAL FELLOWSHIP

The Auckland Division of the Cancer Society of New Zealand (Inc) is offering a Fellowship for post-doctoral research in a field of Chemistry, Cell Biology, or Tumour Biology which is relevant to the interests of the Cancer Research Laboratory, located in the University of Auckland School of Medicine.

Applicants should have, or have almost completed a PhD or equivalent qualification. The Fellowship is available for 2 years with a possible extension for a 3rd year. Salary will be NZ\$18,229 per annum and a return economy class airfare will be paid. Consideration will be given to payment of other expenses, including an airfare for a spouse.

Applications will be accepted up to 1 March 1982.

Further information and application forms are available from: The Chief Executive, Auckland Division Cancer Society of NZ (Inc), PO Box 1724, Auckland, New Zealand. (033)E

#### LINCOLN COLLEGE, Oxford

# EPA CEPHALOSPORIN JUNIOR RESEARCH FELLOWSHIP IN MEDICAL, BIOLOGICAL OR CHEMICAL SCIENCES

The College invites applications from graduates, of either sex, under 28 years of age on 1st October, 1982, for a Junior Research Fellowship in Medical, Biological or Chemical Sciences, tenable for three years from October, 1982; applications from older candidates will be entertained in special circumstances only.

Further particulars may be obtained from the Rector, Lincoln College, Oxford OX1 3DR, to whom applications should be submitted by 14th January, 1982. (013)E

The MOUNT WILSON and LAS CAMPANAS OBSERVATORIES of the CARNEGIE INSTITUTION OF WASHINGTON Announce the availability of THREE RESEARCH FELLOWSHIPS

Applications are invited for Carnegie Fellowships and Las Campanas Observatory Fellowships at the Mount Wilson and Las Campanas Observatories, Pasadena, California, beginning in September 1982. Fellows will be expected to reside in Pasadena, and to devote a major portion of their time to independent research projects. They will have access to the Observatories' research facilities on Mount Wilson, on Palomar Mountain, and on Cerro Las Campanas, Chile. A cooperative arrangement between the Carnegie Institution and the California Institute of Technology permits access to the facilities of Caltech's Palomar Observatory.

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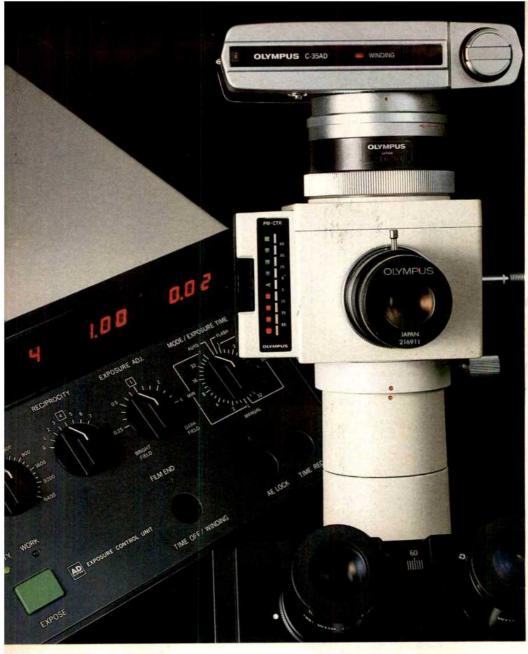
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See page xxiv for a further fellowship

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EMBO Provisional Programme Subject	Organizer and Addre	ess for Further Information and Inquiries	Place Number of Participants
Structure and Function of Invertebrate Respiratory Proteins	Dr. E.J. Wood	Departments of Biochemistry University of Leeds 9 Hyde Terrace Leeds LS2 9LS ENGLAND	19-22 July Leeds 50-60
Functional Integration of Cell Surface and Cytoskeleton Control Systems	Dr. D. Romeo	Istituto di Chimica Biologica via A. Valerio, 32 34127 Trieste ITALY	5-7 April Trieste 60
Formation of Messenger RNA in Animal Cells	Dr. K. Scherrer	Institut de Recherche en Biologie Moléculaire, CNRS Université Paris VII-Tour 43 2, place Jussieu 75221 Paris Cédex 05 FRANCE	28 August-2 September <sup>1</sup> Aroila 80
Cloning of the HLA and H2 Major Histocompatibility Regions	Dr. W.F. Bodmer	Imperial Cancer Research Fund Labs. P.O. Box no. 123 Lincoln's Inn Fields London WC2A 3PX ENGLAND	21-24 March Oxford 60
Replication of Prokaryotic DNA	Dr. E. Veltkamp	Biologisch Laboratorium Vrije Universiteit De Boelelaan 1087 Amsterdam 1007 MC NETHERLANDS	10-15 May Zuidelijk Flevoland 60
Molecular Mechanisms of Brain Development	Dr. J. Nunez	Unité de Recherches sur la Glande Thyroide et la Régulation Hormonale INSERM 78, Ave. du Général Leclerc 94270 Bicètre FRANCE	23-25 June France 50
Membrane Mechanisms Involved in Synaptic Transmission	Dr. S. Algeri	Istituto di Ricerche Farmacologiche 'Mario Negri' via Eritrea, 62 20157 Milan ITALY	5 days June/September Ponte di Legno 50
Intermediate Filaments in Differentiation and Pathology	Dr. M. Osborn	Max-Planck-Institut für biophysikalische Chemie Postfach 968 34 Göttingen FEDERAL REPUBLIC OF GERMANY	23-26 April Günzburg/Ulm 50
Phase Transitions and Conformational Changes in Proteins, Nucleic Acids and Phospholipids: Physical Principles, Mechanisms and Biological Relevance	Dr. HJ. Hinz	Institut für Biophysik und Physikalische Biochemie der Universität Universitätsstrasse 31 84 Regensburg FEDERAL REPUBLIC OF GERMANY	29 March-1 April Regensburg 35-40
Regulatory Mechanisms of Mitochondrial Gene Expression	Professor C. Saccone	Istituto di Chimica Biologica via Amendola 165/A 70126 Bari ITALY	25th June-30th June Near Bari 60-70
Synaptic Connectivity During Development and Regeneration	Dr. I, Parnas	Department of Neurobiology Institute of Life Sciences Hebrew University Jerusalem ISRAEL	4 days in December Eilat 50
Mode of Action of 8-lactam Antibiotics	Dr. D. Vázquez	Instituto de Bioquimica de Macromoléculas Faculted de Ciencias, UAM Canto Blanco Madrid-34 SPAIN	4-10 July Escorial/Madrid 40
Mechanisms of Gonadal Differentiation in Vertebrates	Dr. U. Müller	Institut für Humangenetik der Universität Albertstrasse 11 78 Freiburg FEDERAL REPUBLIC OF GERMANY	5-8 November Freiburg 20
Molecular Biology and Developmental Genetics of <i>Drosophila</i>	Dr. S. Artavanis- Tsakonas	Department of Biology Yale University Kline Biology Tower P.O. Box 6666 New Haven, Connecticut 06511 USA	20-26 June Crete 80
DNA Methylation	Dr. A.P. Bird		4 days in June Nethybridge 48
Organization of Membrane Polarity in Epithelial Cells	Dr. JP. Kraehenbuhl	Chemin des Boveresses	23-27 August Arolla 60 (W524)C



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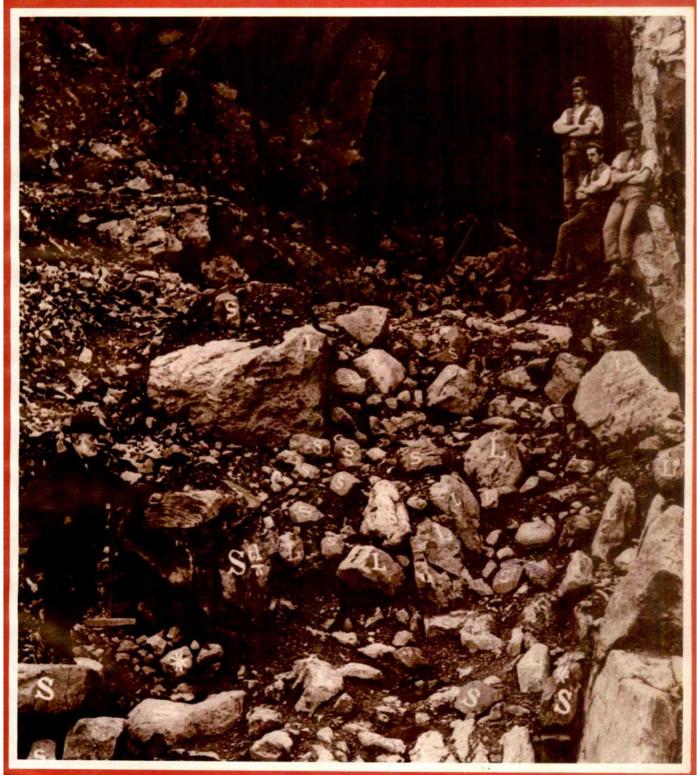
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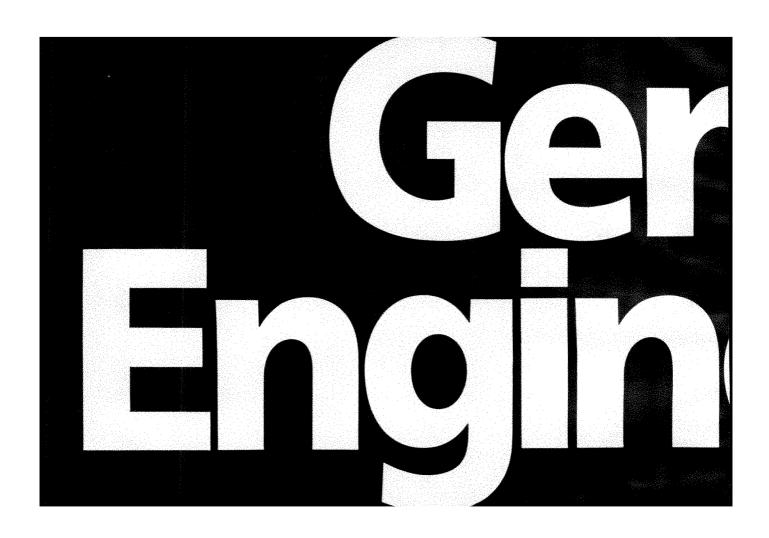
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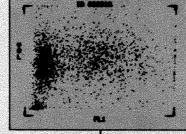
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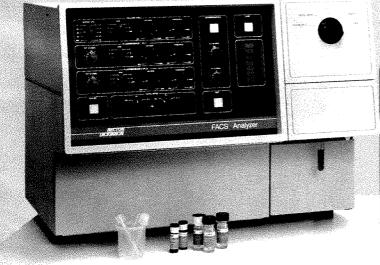
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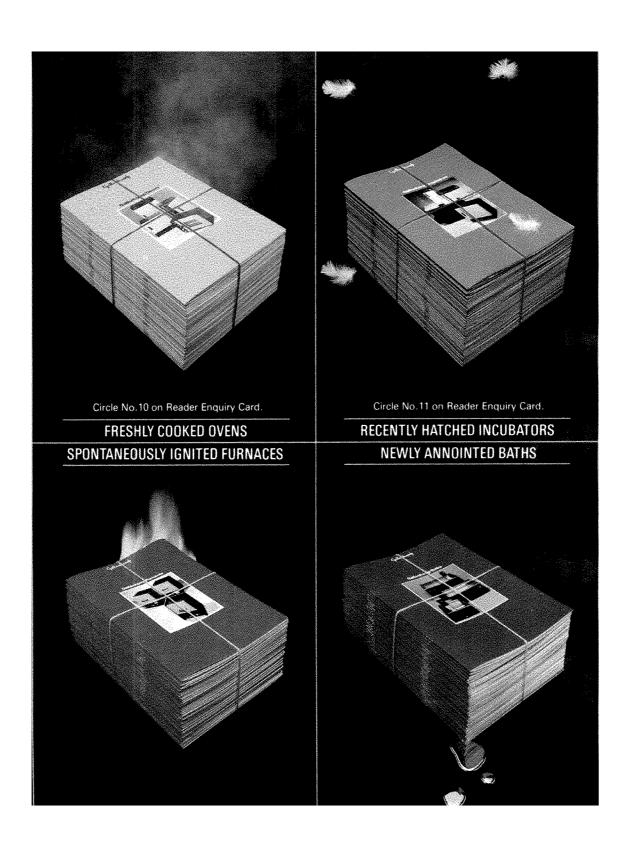
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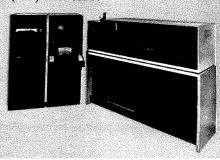
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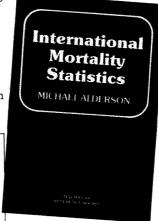
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# nature

## 17 December 1981

# One message on the state of science

The new Presidential Science Advisor last week gave the House of Representatives a vision of how smaller budgets may bring strength. He was partly disingenuous; may he also be right?

The United States Congress has a built-in flair for the theatrical. So much is clear from the circumstances that brought Dr George Keyworth and Dr Frank Press (his predecessor as Presidential Science Advisor now transmogrified to president of the National Academy of Sciences) to the same witness table last week (see page 601). True to expectation and perhaps even to form, Dr Keyworth, the government representative, talked confidently of how his government's policies would make the United States research enterprise more and not less efficient. Dr Press, a little on the defensive, offered a compact between the research community and the United States Administration that would assure basic research in the United States of modestly rising budgets and the United States government of the cooperation of the research community in transferring funds from the "less productive areas or institutes". Both witnesses were talking about the future, naturally enough. Neither of the set speeches mentioned the 12 per cent cut in federal budgets which President Reagan declared in August but which Congress will probably, in the end, decline to give him. But Dr Keyworth's vision was of a future so distant that, by the time it arrives, he may again be an ordinary person not bound to support the policies of the administration of the day.

Whatever the time scale of their dreams, however, Keyworth and Press agreed last week on one essential point — that the federal government has a continuing responsibility for the support of basic research. Keyworth argued that the private sector cannot be expected to support a sufficient effort in basic research, given its short-term need for profit; Press tended to quote President Lyndon Johnson on the subject. One obvious difficulty, which each of them might have mentioned, is that Congress is unlikely to endorse such a fine distinction between basic research — the pursuit of generally unpopular academics and what is called applied research and demonstration. Why should a congressman, never more than two years from his reelection ordeal, vote for the general distribution of research funds to the generality of academics when he could vote instead for his own state's favourite federal laboratory or demonstration project? Dr Keyworth should have been prepared to remind Congress of its own responsibilities. Dr Press would no doubt have been prepared to back him up with accounts, gathered at first hand, of how the pork barrel rolls.

For the immediate future, the outstanding question is whether the scientific community is in a position to do what Dr Keyworth asks of it, and to use the system of peer-review committees, and of the advisory committees that advise the grant-making and mission-oriented agencies respectively, in such a way as to concentrate funds for basic research in fields of enquiry and on institutions that are likely to make best use of them. Broadly speaking, the answer is that it all depends. For Press is also right to imply in what he said last week that the research community can do this kind of job when it believes that it can do so safely, knowing that its paymasters will not take its recommendations of places or people from which funds can be withheld as licences to go ahead while saying that recommendations of where support should be increased can wait until the economy has improved. Dr Keyworth's speech last week, full though it was of references to a creative basic research community x years from now, conspicuously lacked a sense of responsibility towards — or even regret for - those who have contributed to the same endeavour in

the past x years. Keyworth was looking not at the future but at the horizon beyond it.

Press's recipe for the immediate future is, unfortunately, equally unpalatable. Faced with present economic uncertainties, the United States government could not agree to a compact that would assure basic research of some specified rate of growth each year. The economic crisis that afflicts science in the United States (and elsewhere) has arisen precisely because people are uncertain about the economic future. Other institutions are similarly afflicted. None can be insulated. Keyworth last week would have been wrong to promise that they might be. The best course for him and Dr Press for the weeks ahead will be to make plain that nobody is owed a living, but that the government will do what it can, within the resources at its disposal, to make the best use of what can be spent on basic research.

Two minor scandals stand out from last week's exchange. Dr Keyworth argued in his statement that things may not be too bad because the Pentagon's spending on basic research will be increasing in the years ahead. One trouble is that what the Department of Defense counts as basic research is not always what other people understand by the term. Another is that the Pentagon's sense of what is excellent is often compromised by its sense of what may be expedient. If the traditional and trusted sources of support for basic research are to be cut back but the Pentagon enlarged and the research community as a result given less of a say in what is excellent and worthwhile, should not Dr Keyworth have been urging that this year's increase in the Pentagon's operating budget should be matched by a corresponding increase of the budgets of the agencies dedicated to (and skilled at) providing support for basic research?

The second scandal that will stick in people's minds is Dr Keyworth's homily last week about the neglect of science and mathematics in American high schools. Since the post-sputnik era in the late 1950s, enthusiasm for the physical sciences has evidently waned. Rightly, Dr Keyworth shares with other observers a sense of anxiety about the consequences. Will United States universities in future be providing remedial mathematics as freely as they now have to offer remedial English? Or will it simply be that the brightest young people follow different kinds of courses, letting American industry depend on skilled people recruited from abroad? The fears may be exaggerated, but Dr Keyworth's defence of his government's inaction is equally an exaggeration of the doctrine that Republican governments are ideologically opposed to intervention in other people's affairs. Keyworth said that the Administration is concerned at the decline in high school studies in the physical sciences, but that education is a matter for state and local government and that he had confidence that, sooner or later, they would see the light. Can he be sure, especially when the government for which he works has decided (as it did in its March budget) that the National Science Foundation should be effectively robbed of funds for the further development of the science curriculum? If the benefits of basic research are too distant to be left to industry, can it safely be assumed that the long-term needs of the educational system can be entrusted to embattled school committees? And if not, does not the Administration have a duty to show the way? The National Science Foundation's educational programme may have aged ungracefully, but the Administration cannot wash its hands of the set of problems its science adviser has identified.

# Finniston's pale shadow

The British Engineering Council now exists. Its first task is to define for itself a role.

Engineering education promises to be in the 1980s a lightningrod for public solicitude to take the place occupied in the 1950s by the cause of motherhood. In the United States last week, Dr George Keyworth was wringing his hands before Congress in anxiety about the difficulties of recruiting and then keeping members of university engineering faculties — but was confident that the market would somehow find a way. In Britain, engineering education has been a public issue for at least four years, since Sir Monty Finniston's committee was set up. Broadly speaking, there have been three distinct complaints. First, there is not enough engineering education. Second, what there is is either inappropriate or not good enough or both. And third, in universities as in the world outside, engineering has a lower status than science. Finniston's report (now nearly two years old) embraced all these views and more, and urged that there should be an Engineering Council equipped with powers over engineering curricula in all kinds of teaching institutions and with the funds with which to implement reforms. Implicitly, Finniston asked that his council should supersede the existing engineering institutions, some of them monuments to the Industrial Revolution.

The contrast between the United States and Britain is instructive. In both countries, it is acknowledged that engineering skill is one of the mainsprings of industrial innovation, productivity and prosperity. There, however, the similarity ends. Dr Keyworth last week was merely echoing the longstanding complaint that American industry is so fully seized of the economic importance of engineering skill that universities cannot easily compete for teachers — and graduates flock off to lucrative jobs in industry undiverted by the prestige of the academic life. In Britain, much the same may be happening in fields of engineering linked with computer engineering, but for the most part those carrying a torch for British engineering are still mouthing hackneyed slogans — that engineering (and engineers) should be accorded more "status" by everybody in sight.

This is the spirit in which the Committee of Engineering Professors has in the past few months been urging that the Science and Engineering Research Council should be split into two, one part concerned with the support of engineering research in universities. For, the argument seems to be, how can engineers respect themselves without a research council to call their own? (The proposal, mercifully, seems to have been headed off by the willingness of what used to be the Science Research Council to mention engineering in its title.) Similarly, there is excited talk, from people such as Lord Caldecote, the president of the Engineering Fellowship (another pressure group), that the new council may help to give the engineering professions public esteem. What these complainants consistently overlook is that the exercises in public relations on which they are embarked are merely palliatives, and that British engineering would be better off if its esteem were reflected in the eagerness of British industry to snap up young engineers — and to pay them decent salaries.

How is that to be accomplished? And will the new council help? Much will depend on its willingness to come to grips with the educational questions which Finniston grasped too firmly — like a man picking up a stinging nettle. In spite of the inquiries of the past four years, nobody is much wiser about the balance that should be struck between the various ingredients in various kinds of engineering courses. Instead, at the university level, the assumption is being established that there should be two kinds of courses, one lasting three years and one for four.

Unfortunately, very little thought has been given to the academic content of these courses, and the suspicion remains that university engineering courses, like those in other educational institutions, are too narrow because the separate engineering institutions too jealously insist that courses should concentrate on the topics judged to be relevant to their fields of interest.

Mechanical engineers worry about the design of gear wheels, civil engineers about the design of bridges, electrical engineers about the design of circuits and so on. The influence of the engineering institutions stems from their willingness to grant exemption from some of the requirements for professional qualification to students who have followed approved courses. This right will remain under the new arrangements. But who is to say that these are the lines on which engineers should be taught? Might not a preparation in something called engineering science be a better preparation for work in a profession that must turn its hand to almost everything? And how is the engineering profession to come to terms with the disconcerting truth that many whose academic training is quite different from that now required of engineers still function in industry as engineers? It would be splendid if the new council chose, as its constitution allows, to license the occasional physicist or chemist to practise as an engineer. Better still, it should take a fresh look at what academic preparation is needed by those who may in future keep British industry alive. But it should firmly acknowledge that a demand for more prestige for engineers is tantamount to a demand that water should run uphill.

# Who pays what piper?

British universities and football clubs are in trouble. Can they make common cause?

Economic crisis is at least even-handed. Last week, British newspapers were full of gloomy prophecies of how no fewer than a dozen among three-score British institutions are threatened with bankruptcy in the months ahead. The causes are easily described. Running costs have risen. The cost of employing staffs has become doubly onerous - the annual salary bill is high, but the longer-term implications of people's contracts with their institutions are a shadow over the future. But income is falling. Traditional customers can no longer afford to take advantage of the services the institutions exist to provide. Inevitably, where the threatened institutions command particular loyalty from the cities in which they are based, especially when they are the only one of their kind, bands of loyal supporters have formed to conjure a phoenix from the impending ashes. Elsewhere, newspaper columnists comfortably bemoan the passing of a revered tradition and a past source of public pleasure and enlightenment. The institutions are the professional (commercial) football clubs that play with a round ball, otherwise known as soccer or "association football' clubs.

British academics reading this torrent of gush (which may be made meaningless if only the clubs can negotiate a better television contract next season) may be forgiven for not recognizing that much of the purple prose could apply to the institutions for which they work. The proportions at risk are about the same. The inexorable increase of expenditure has similar causes. And income is falling because the government, effectively the only customer, cannot afford to pay for the services on offer. But may not academics, most of whose institutions will be making critical decisions about their future this week and next, learn from what the football clubs are planning?

Many football clubs plan to make better use of their fixed plant; universities might similarly offer courses out of business hours or in vacations. Other clubs plan to increase their ancillary income; selling franchises to put university coats of arms on tee-shirts would hardly be lucrative, but what of the benefits from academic enterprises with which British univesities have so far only flirted? Some clubs plan to sell off players, presumably the most saleable and thus probably the best; but universities are thinking of offering early retirement only to the least employable. No football club lacks a local constituency, a group of people to which is has given pleasure in the past and which is now prepared to fight that it should have a future; too many universities, unfortunately, are unable to make a comparable claim on their localities. In a macabre way, it will be interesting to see which threatened group does best in the months ahead.

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# Keyworth and Press on basic science

# Researchers to decide where funds go?

Washington

In the first coherent statement of the Reagan Administration's thinking on science policy, Dr George (Jay) Keyworth, the president's science adviser, said last week that the scientific community must be prepared not only to help identify areas in need of increased support, but also to say where support should be decreased.

The time of constantly growing budgets across the board had passed, Dr Keyworth said, and the scientific community's "best and most thoughtful judgement" was needed to identify areas to deemphasize as well as those meriting new emphasis. "To those who object to such undertakings, and to all of my scientific colleagues, I must say that if scientists do not make such choices, others will, but with less acuity."

Dr Keyworth was addressing a hearing of the House of Representatives Science and Technology Committee on the implications for science of the present budget reductions. At several points in his testimony, Dr Keyworth claimed that the Reagan Administration was promoting a fundamentally new approach to the government's responsibilities for science and technology. "I am proposing a federal role in research and development which is appropriate to the 1980s — appropriate to a national mood which calls for increased vigour and acceptance of responsibility by individuals and organizations in the private sector and decreased involvement by the federal government in many of our affairs", Dr Keyworth said.

Yet apart from promoting the need to trim research budgets to fit straitened economic circumstances, much of the substance of what Dr Keyworth had to say was similar to statements to the committee made by his two predecessors, Dr Frank Press, science adviser to President Carter and Dr Guyford Stever, adviser to President Ford. All three stressed the federal government's responsibility to promote basic research as a long-term investment in the future. And all agreed that short-term research and development should primarily be the responsibility of the private sector.

Dr Keyworth, in line with the overall policy of the Reagan Administration, emphasized that science policy had to be carried out in the context of other national policies such as national security, international relations, energy, social services and the economy. "For example, science policy, made without considering econo-

mic policy, is irrelevant", he said.

Dr Keyworth's immediate predecessor, Dr Frank Press, now president of the National Academy of Sciences, while accepting the need for financial restraint, gave last week's committee hearing a sharply different view. He advocated a ten-year "compact" between government, industry and universities to establish new national goals for the support of science. This would include commitment to an annual real growth of between 1 and 2 per cent in the scientific research budget; an additional 1 per cent growth to support special "targets of opportunity" in different fields; and a real growth of 1 per cent, equivalent to about \$50 million a year in the contribution made by industry to university research.

To a large extent, however, the differences in the statements from Dr Keyworth and Dr Press were a reflection more of their different constituencies than of disagreements on substance. Both agreed that there should be a reduction in federal support for "demonstration projects" that should properly be taken over by the private sector. And Dr Press spoke of Dr Keyworth's "courage" in

making uncomfortable decisions about what research projects should be cut.

Responding to a question about press reports that he had recommended the elimination of all new planetary exploration missions by the National Aeronautics and Space Administration (NASA) over the next decade—a proposal being discussed this week by President Reagan and NASA administrator James Beggs—Dr Keyworth emphasized the many scientific gains to be made from space shuttle missions, and suggested that this was one area in which direct scientific comparisons might be made.

Expanding on a theme of several of his earlier speeches, Dr Keyworth told the committee that there were several reasons why the United States could not expect to be preeminent in all scientific fields. He also warned against the tendency to avoid tough decisions about which projects should be cut by applying cuts uniformly across all fields. "I believe the discipline of making such hard choices will ultimately benefit science, just as the occasional pruning of a tree can promote, rather than retard, its heal."

David Dickson

### Spain has the money but not the people

Science in Spain seems to be embarked on a period of rather heady growth. Money under the direct control of the Spanish science research council, the Consejo Superior de Investigaciones Cientificas (CSIC), will triple next year if decisions taken by the lower house of the Spanish parliament are confirmed by Senate. This comes on top of a doubling last year of the centrally administered "funds for research", from which CSIC draws the largest single portion. One sign of how the wind is blowing is the story of a Spanish chemist, recently returned from the United States, in a poorly-equipped CSIC laboratory. In the past few weeks he has found himself spending money "eight or nine hours a day" bringing his laboratory up to scratch; and he has not even had to submit a formal grant application.

CSIC plays a major role in science in Spain, maintaining 23 institutes in the humanities and 77 in natural sciences and employing 1,600 scientists plus 4,400 supporting staff. But the Consejo is not the only beneficiary of increased spending. Parliament has also agreed to create two new research funds, on which universities (and others) will be able to draw: one to be controlled by the Ministry of Industry, and one by the Ministry of Health, together increasing the government research budget by about a quarter.

The regional distribution of support is also becoming a shade more equitable. Whereas some 85 per cent of the central funds for research have usually been spent in Madrid, parliament this year has insisted that 25 per cent be spent in the regions; and the autonomous government of Catalonia is also expected to increase its fledgling research budget next year, from 100 million pesetas (around £500,000) to 200-250 million pesetas.

In total, over the past two years Spanish spending on research and development has nearly doubled as a fraction of gross national product from around 0.3 per cent to 0.5 per cent now — still way below the developed world average of something over 2 per cent but an increase which is already proving difficult to manage.

The problem is people. The truly effective Spanish research community is very small, and there is a government freeze on new appointments. This is having two serious effects, which some feel must be corrected quickly or the new money will be merely wasted.

First, there are not enough highly qualified scientists to act as critical referees in all fields in which Spain would wish to do research; although arrangements have been made for refereeing all proposals, in practice the judging has proved to be imperfect and partisan. An international refereeing system is proposed by those who would survive it; the others — inevitably the majority — resist.

Second, there are too few posts becoming vacant to employ the rising tide of experienced Spanish scientists who wish to return from other countries and make use of the new money. So paradoxically the coffers are open, but there are good

scientists unemployed.

The man who may change all this is the new minister for universities and research, Professor Federico Major, recently returned from Paris after a spell as Deputy Director-General of UNESCO. Major, a 47-year-old developmental neurobiologist, has kept in touch with science despite a long career in politics. He is a personal friend of King Juan Carlos, and yet is right wing (he was minister of universities under Franco) and so may be able to ride the strong conservative opposition in Spain. He has to steer through a new law for the universities (now entering its seventh draft). And he appears to have been impressed, during his time in Paris, by the new French commitment to science as a means of economic development. He is already talking of a "law for science" which would define a budget and a programme for a stretch of a few years, so clearing all political hurdles in one jump. This is exactly the strategy of the French minister for science. It will be interesting to watch how far Professor Major will mimic him.

Robert Walgate

#### UK biotechnology

### **Blood money**

A British publicly-funded organization for encouraging innovation in industry has made its second major investment in the fledgling biotechnology industry. The British Technology Group (BTG — an amalgamation of the former National Research Development Corporation and the National Enterprise Board) announced this week that it is investing £2 million in Speywood Laboratories Limited of Nottingham for the development of new techniques for blood protein manufacture. Prutec Limited, a subsidiary of the Prudential Assurance Company, will be matching BTG's investment.

BTG's other biotechnology venture is Celltech, the company it established last year with three finance houses and the Medical Research Council to develop recombinant DNA techniques for manufacturing medical products, including monoclonal antibodies. The group's latest venture is to develop a new fractionation technique for manufacturing blood proteins, an area in which Celltech is not involved.

Speywood, a small company set up seven years ago, will use the £4 million roughly to double the size of its factory and research laboratories. Its initial aim is to improve its polyelectrolyte process for fractionating blood cryoprecipitates, which allows the separation of a greater variety of blood proteins than the traditional Cohn process for fractionating whole-blood plasma. The company has already used the new technique for isolating a pure form of factor VIII from pigs' blood for use in particularly sensitive human patients. Clinical trials are expected next year. It now plans to expand factor VIII

production and extend the technique to factor IX, factor VWF (for treatment of von Willebrand's disease) and fibronectin.

Speywood is the only commercial company now producing blood products in Britain, the bulk of production being controlled by the health department through its Blood Products Laboratory at Elstree, and Speywood's plans for expansion are likely to be welcomed by the National Health Service, which has to import a large proportion of its supplies.

Speywood's longer-term goal, however, is to develop and use recombinant DNA techniques for manufacturing blood proteins such as factors VIII and IX, fibronectin,  $\alpha_1$  antitrypsin and albumin.

David Heath, managing director of Speywood, welcomes investment from BTG and Prutec not only for the money but also because it gives the company access to expertise on recombinant DNA technologies in universities (BTG acts as a "broker" between industry and the universities). He hopes that the £4 million will launch the company into an expansion plan costing about £19 million over the next five years. Later on, he will be looking for further investment, but BTG is for the time being non-committal, preferring to see how the company shapes up before committing itself further. Judy Redfearn

#### **Telecommunications**

# French hanging on

Brussels

French doubts about the wisdom of opening up some public purchasing contracts in telecommunications to its partners in the European Economic Community held up agreement last Monday on a set of recommendations designed to stimulate the growth of the European market in telecommunications.

Although the recommendations would not be binding, they constitute a first step towards achieving community-wide services and a community-wide market for terminal and other kinds of equipment. The telecommunications administrations of the various member countries have, of course, been cooperating for a long time but the supply of equipment for national networks has tended to remain in national hands. Following the meeting of the Council of Telecommunications Ministers in December 1977, the European Commission established a working group on future networks which has recommended urgent action in the field of digital networks. But the different policies being pursued and conflicting commercial considerations continue to hamper progress.

The first recommendation is for consultation with a view to ensuring that new services are introduced within the community only when they are mutually compatible. Second, the telecommunications administrations of member

#### Pleasant surprise

The shortfall in admissions of overseas students to British universities has not been as great as anticipated. According to the University Grants Committee (UGC) admissions of overseas undergraduates in 1981-82 totalled 4,918 compared with 5,017 in 1980-81, a shortfall of about 2 per cent. Postgraduate admissions were 7,414, only 0.5 per cent less than in 1980-81.

These figures, however, come on top of reductions in 1980-81 compared with 1979-80. The sharpest reductions then were in students from the United States and Malaysia which sent 1,105 and 3.988 undergraduates respectively, compared with 1,450 and 4,188 in 1979-80. Most other countries cut back by two to five per cent. The exceptions were West Germany, Hong Kong and Nigeria which sent more students than in 1979-80. The largest contributors of overseas students are still Malaysia, Hong Kong and the United States, between them sending more than half of Britain's total overseas enrolments.

As yet UGC has no figures for individual universities or for the countries of origin of new entrants for 1981-82, but the unexpectedly small decline should mean that some universities, at least, have not suffered as severe a loss of in income from overseas students' fees as had been feared. The effect may be to lessen the impact of the government's cut in the university grant.

Judy Redfearn

countries should not discriminate between domestic and other EEC suppliers of telematic terminal equipment by means of type approval procedures taking longer than six months, or which are more complex and costly to comply with than those employed elsewhere. But it was the third recommendation which caused the greatest problems. This specified that for the period 1981–83, telecommunications agencies should seek tenders from suppliers who manufacture in other community countries for at least 10 per cent of their annual orders.

The French quibbled about the phrasing "suppliers who manufacture", partly because, as one diplomat put it, "they don't want to go ahead with the recommendations anyway". But the French also want to avoid loopholes that will open up their market to non-EEC countries. The worries of the Germans, the other main objectors to the recommendations, were, however, removed. The Bundespost's ambitious videotext programme is being accompanied by a new law to extend its monopoly in new information systems and terminals such as modems, although this move will be examined by the European Commission's directorate on competition.

Jasper Becker

#### Chemical health risks

### Not so great

Washington

After reviewing the scientific literature on the health risks associated with the use of nitrites and nitrates as food preservatives, a committee of the National Academy of Sciences National Research

#### Backing a winner

One of the few European research facilities which can claim a substantial lead over the United States in both size of budget and research quality, the high flux neutron beam laboratory at Grenoble (Institut Laue-Langevin, ILL), now has the money and agreements it needs to keep ahead of the opposition until the 1990s, its directors believe.

In the past two weeks two major decisions affecting the future of ILL, which provides services to 1,700 visiting scientists each year, have been taken. The treaty between France (the host country), Germany and the United Kingdom, which guarantees support for ILL, was extended a further 10 years to 1992; and the international steering committee, which decides the budget and a five-year forward look each December, has agreed to a substantial addition to the neutron source costing some £2 million, and an increase in the number of major instruments at Grenoble to make use of it.

The improvement — a new "cold source" which produces neutrons at long wavelengths for large-scale structural studies such as of polymers or of biological materials — was the principal part of a large programme approved in outline in 1979; but there were fears that cuts in German and British budgets might threaten it. These fears have now proved unjustified.

ILL directors see the new source, basically a tank of liquid deuterium which cools thermal reactor neutrons to low velocities and hence long de Broglie wavelengths, as a means of capitalizing on ILL's greatest successes (which have been in long-wavelength scattering) and of countering — or complementing competition from accelerator-driven 'spallation' neutron sources. These may provide improved flux and resolution at short wavelengths by making use of spallation reactions, where incoming protons (from an accelerator) collide with heavy neutronrich nuclei (such as uranium) to produce a spray of neutrons ten to one hundred times the intensity of the incident proton beam. Spallation sources are planned in Japan, and at Argonne and Los Alamos in the United States; and one is under construction at the Rutherford Laboratory in the United Robert Walgate Kingdom.

Council (NRC) has concluded that current exposure levels constitute an insufficient risk to require major reductions in their use.

However, because many nitrosamines and other nitroso compounds which can be formed from nitrates and nitrites have been found to cause cancer in laboratory animals, and can therefore be associated with cancer in humans, the committee has recommended some reductions.

The committee was set up by NRC at the request of the US Department of Agriculture and the Food and Drug Administration to study the health effects of nitrate, nitrite and N-nitroso compounds, following the publicity which occurred in August 1978 when workers at Massachusetts Institute of Technology found that sodium nitrite caused tumours in the lymph systems of rats.

At the time the federal government rejected calls for a temporary ban on the use of nitrites as a food preservative, but established an immediate study of the problem. In August 1980, the two agencies announced that they had found "insufficient evidence" to link the use of nitrites to cancer but said that they intended to study the issue further.

Last week's report is the first of two to be produced by NRC in response to the government's request by a panel chaired by Dr Maclyn McCarty of the Rockefeller University in New York. The panel's second report will consider current research and prospects for developing alternatives to nitrite as a food preservative.

In its first report, the committee says that the results of limited experiments suggest that nitrate is neither carcinogenic nor mutagenic, but that evidence from several epidemiological studies in human populations is consistent with the hypothesis that exposure to high levels of nitrate may be associated with an increase of cancer of the stomach and oesophagus, recommending further studies to confirm these preliminary findings.

The committee also says that scientific evidence does not indicate that nitrate acts directly as a carcinogen in animals. In contrast it says that most N-nitroso compounds are carcinogenic in laboratory animals, mutagenic in microbial and mammalian test systems, and that some are teratogenic in laboratory animals. However, it adds that such results are of limited value for predicting the quantitative risk to humans.

At the same time, it points out that nitrites found in cured meats account for only a small proportion of the total exposure to nitrosamines.

According to the committee, cigarettes represent the single largest source of nitrosamines, with a daily pack of American filter cigarettes producing an exposure of 17 microgrammes. In contrast, the level ingested from all dietary sources is about 1.1 microgrammes a day.

**David Dickson** 

#### Satellite launcher market

### Ariane set fair?

The European commercial satellite launcher business is itself well launched. With two successful test flights to its credit, Ariane, the European Space Agency's heavy satellite launcher, is already qualified for service. So whatever the outcome of the fourth and final test flight this week, the fifth launch next March will be the first of six promotional flights, after which production and marketing of subsequent launchers will be officially handed over to the French-based company Arianespace. But the space agency's role in rocket development continues. By 1983, Ariane 2 and 3, capable of placing 2,100 kg and 2,580 kg payloads into geostationary transfer orbit, will be in service.

Further ahead, in 1985 there will be a test launch of Ariane 4, which uses the basic design of Ariane 3 but is to be offered in six versions, with payloads of 2,300–4,300 kg, achieved by strapping onto Ariane 3 various combinations of liquid and solid-propellant boosters. This more ambitious version of Ariane 4 has been prompted by calculations of the mass of the telecommunications statellites likely to be built towards the end of the decade. The European Space Agency's swift approval of this version of Ariane 4 is evidence of its determination to compete internationally in the growing market for satellite launchers.

The only other present source of commercial launching facilities is the United States National Aeronautics and Space Administration. The Thor Delta and Atlas Centaur launchers can put 900 kg and 1,850 kg into geostationary transfer orbit respectively, compared with the current capacity of Ariane 1 of 1,700 kg. Although the shuttle will be capable of carrying much heavier payloads, Europeans are quick to say that it is technically unproven and that availability is still in doubt.

Meanwhile, there is little competition for Ariane from elsewhere. The Soviet Union has geostationary launch capabilities of 2,400 kg and 5,000 kg aboard Soyuz and Zonda, but these are not generally commercially available. Thus the most likely competition in the future will come from Japan, which already has a small rocket capable of launching 220 kg into a low circular orbit and is developing a heavy satellite launcher, which could rival Ariane, for a first launch in 1987.

The only other contenders so far are China, India and Brazil. China and India have already launched small scientific payloads into near-Earth orbits. The immediate goal of the Indian programme is to develop a Polar Satellite Launch Vehicle by the mid-1980s for putting meteorological and remote sensing satellites weighing up to 600 kg into near geopolar orbits which pass over all points on the Earth at the same time of day. Only then will attention be turned towards a launcher

for heavy communications satellites.

Brazilian plans are more tenuous. For a decade, the government has been talking of launchers to place remote sensing and meteorology satellites into orbit, though little seems to have been done.

Judy Redfearn

#### Polish science advice

#### Reforms in limbo

One of the casualties of the Polish government's sudden suspension of the Solidarity trade union at the weekend may well be the draft bill that would have made it obligatory for government agencies to consult "scientific experts" before making important decisions. News of the bill came in a communiqué issued last month after a meeting between the Prime Minister, General Wojciech Jaruzelski, the Primate of Poland, Archbishop Jozef Glemp and Lech Walesa, leader of Solidarity.

To be effective, the proposed bill would have had to ensure that consultations were more than an empty formality. In some cases it would seem, past governments acted without even an appearance of consulting scientists. A notorious case was the siting of vast industrial plants — the Skawina aluminium smeltery and the Lenin steel mills — just outside Krakow. The decision to develop industry in the area in the 1940s had political overtones but meteorologically Krakow is a very poor site because of the limited circulation of the air. The toxic fumes from industry have undermined the health of the population and made the produce of farms unsafe within a radius of 50-60 km.

Until September 1980, any public discussion of such issues was impossible. Within a month of the signing of the Gdansk accords, however, a new Club of Polish Ecologists was established, based in Krakow, which early in January succeeded in having the obsolete production lines at Skawina (which had been emitting hydrogen fluoride) closed down. General Jaruzelski has been a strong supporter of moves to give scientists a louder voice, and he seems committed to continuing with consultations. But exactly who will be involved in any future talks is now unclear, as a new format is likely to emerge after a brief hiatus.

A second new bill now being prepared is aimed at reforms in the Academy of Sciences. The academic secretary of the academy is at present directly responsible to the prime minister and has himself quasiministerial rank. In recent months there has been a considerable movement within the academy to change this anomalous status by making the academic secretary responsible only to his fellow academicians. It seems likely that the prevailing "state of emergency" will mean a considerable weakening of provisions in the bill aimed at increasing the independence of the academy. Vera Rich

#### Creation on trial

# **Battle engaged**

Washington

While a federal judge in Little Rock, Arkansas, listened to the closing arguments this week in a case claiming that a new state law which requires the teaching of "creation science" violates the separation of church and state, both sides in the dispute were already sharpening the arguments for the next round of what promises to remain an escalating battle of wits.

In Washington, the National Academy of Sciences held the first meeting on Monday of a committee made up of prominent scientists and legal advisers who will prepare a legal brief on the scientific status of the theory of evolution to be presented by the academy as an *amicus curiae* ("friend of the court") document, either to the Arkansas court if there is enough time, or in any future legal proceedings.

Meanwhile the creationists are working on a revised version of their "model bill" used as the basis of the Arkansas law passed in March, requiring equal efforts to be devoted to teaching the theory of evolution and creation science in school biology classes. The new bill is designed to meet some of the legal challenges thrown up in the Little Rock proceedings and elsewhere.

Whichever way the Arkansas verdict goes, there is a good chance that the fight will make its way up to the Supreme Court. And the supporters of creation science are unlikely to be put off by an adverse legal ruling, since they claim to be backed by a groundswell of popular support.

The academy panel is being chaired by Dr James Ebert, vice-president of the National Academy of Sciences and president of the Carnegie Institution of Washington. Others on the panel include Dr Steven Weinberg, professor of theoretical physics at Harvard; Dr Preston Cloud, professor of biogeology at the University of California, Santa Barbara; and Professor Norman Newell, curator emeritus in the department of invertebrates at the American Museum of Natural History in New York.

Several legal experts have been included on the panel to advise on the constitutional issues raised by the creationists. These include Mr Peter Barton Hutt, previously general counsel of the Food and Drug Administration, and Dr Richard Maserve, a staff member of the Office of Science and Technology Policy under the Carter Administration. Both are now members of the Washington law firm Covington and Burling.

The legal brief will concentrate on providing an academy-endorsed statement on the definition of science which, it is hoped, will help both courts and state legislatures distinguish the philosophical status of the theory of evolution from creation science.

The academy is also considering

producing a booklet to summarize current thinking on evolutionary theory.

The main claim being put forward by the state attorney general, Mr Steve Clark, in Little Rock is that, since holes can be picked both in the theory of evolution as a conventional science and in creation science as a conventional religion, the two are "just as scientific" and "just as non-religious" as each other.

The American Civil Liberties Union (ACLU), which has filed the case against the state of Arkansas on behalf of several local religious groups and school teachers, has so far had no difficulty in generating substantial support, from both the legal and the scientific professions, in preparing its case. A prominent New York law firm for example has been providing free legal support — including extensive research and the services of eight back-up attorneys in Little Rock — which would normally cost many hundred thousand dollars.



"Next witness"

ACLU has received help and advice from between 60 and 70 scientists in preparing a brief. Among those called to the witness box who gave a vigorous defence of evolutionary theory and challenged the claims of creation science were Professor Francisco Ayala of the department of genetics at the University of California in Davis, a member of the new National Academy of Sciences committee; Dr Gary Dalrymple, a geologist who is an associate director of the Western region of the US Geological Survey; and Harvard evolutionary biologist and historian Dr Stephen Jay Gould.

In contrast, although the state attorney general's office is presenting a number of scientists to put the creationist interpretation of human origins, few have significant standing in the scientific community. Attorney General Steve Clark complained last Thursday that several scientists had refused to testify in defence of the new law, suggesting that they had been subject to "peer group pressure".

Not all creationists have been happy with the way that their case is being handled. Two of the more prominent attorneys associated with the creationist movement, constitutional expert Wendell Bird and Virginia attorney John W. Whitehead, complained that they had been excluded from playing a major role in preparing a defence of the law, and that the state had therefore prejudiced its own chances of defeating the law suit.

Both, however, are now preparing for the legal battles that lie ahead. And even if ACLU wins in Little Rock — as seems likely, given the liberal background of Judge William Overton and the way he has handled the case so far — next time round the arguments could be tougher to defeat.

One passage in the Arkansas bill, for example, speaks of creation science being based on the explanation that the world was created ex nihilo, a passage which theology professor Langdon Gilkey of the University of Chicago described as "the most religious" of various statements implying the involvement of a God, since "there are no other sources at work". The new version of the model bill, however, which is being circulated by Mr Paul Ellwanger of Citizens for Fairness in Education of South Carolina, merely states that creation science must be based on 'evidences that indicate creation of the Universe, matter and energy suddenly".

Given the fact that challenges from creationists are unlikely to evaporate, even if they lose the Arkansas case, ACLU is asking the judge for a "finding of fact" that any science must be based on natural laws and must be explanatory, falsifiable and tentative, criteria which they hope will rule out creation science as a genuine science, however it is described.

**David Dickson** 

#### UK cancer research

### **Unequal shares**

If the Medical Research Council's (MRC) reshuffle in the allocation of funds for cancer research in Britain was intended as a bid to save money, it has been largely unsuccessful. The complete withdrawal of financial support from two cancer research institutes in Manchester and Glasgow has saved the council a paltry £0.5 million.

Instead, it seems, the changes - announced last month in the council's annual report — were aimed at a rationalization of administration in the Patterson and Beatson cancer research institutes in Manchester and Glasgow. Since 1970 the two independent laboratories have been financed by a joint committee of the Cancer Research Campaign (CRC) and the MRC, with the two bodies taking equal financial responsibility. "The MRC's decision to relinquish financial commitment to the CRC," says Dr John Paul, director of the Beatson laboratories, "has made administration here much easier. Working for two paymasters can become complicated.

The Beatson and Patterson laboratories may benefit from dependence on the publicly-supported CRC, but the Institute of Cancer Research in London (ICR) is left as pig-in-the-middle. It had been hoped

#### Painful adaptation at cancer institute

The problems of the British Institute of Cancer Research (ICR) stem largely from the events of four years ago, when its funds were substantially cut and put on a basis unusual for a British institute. The block grant to ICR has been tapering away for the past few years. From next April, research groups will have to compete exclusively for funds with other applicants to the Medical Research Council and the Cancer Research Campaign. To make matters more complicated, block funding of laboratory services has now also been withdrawn so that, for example, a research group which needs electron microscopy is now expected to obtain sufficient funds to pay for it as a properly costed service; consequently ICR will be able to maintain an electron microscopy facility only if there is sufficient demand and cash from the research units to support it continually.

Worse still, in the period between the sudden early retirement of the previous director of ICR, Dr Thomas Symington, in August 1977 and the appointment last year of Dr Robin Weiss, little had been done to adjust to the new circumstances. Indeed, ICR had gone on spending almost as though its total funds had not been cut by about 18 per cent, with the result that Dr Weiss inherited a deficit of about £1 million even though the Imperial Cancer Research Fund had donated about the same amount to help stave off the day of reckoning.

Since his arrival, Dr Weiss has had to clear the £1 million deficit and adjust to an annual budget that is now about £6.5 million — £4 million from the Medical Research Council and the Cancer Research Campaign, the rest from legacies, the National Health Service, endowments and granting bodies. About £400,000 a year has been saved by early retirements, the freezing of

vacancies and other means — for example, ICR no longer has laboratory cleaners. (Has anybody noticed?) About 45 members of staff have gone, a loss offset because Dr Weiss, with separate funds linked with his appointment, has brought in about 20 new people to establish a core of molecular and cellular geneticists.

But now, Dr Weiss and his newly appointed deputy director, Dr Tony Davies, have to find more savings. Unless some means can be found to spread the contraction over four years or so, by which time more natural or voluntary retirements will have done the trick, it looks as if there will have to be perhaps 50 compulsory redundances.

One immediate threat is to the Division of Tumour Immunology under Professor Peter Alexander at the Sutton branch of ICR. As a result of recent site visits, the Medical Research Council and the Cancer Research Campaign have very substantially cut the funds of this division, leaving uncertain the future of all the tenured staff.

Dr Weiss is not convinced of the justification of this drastic cut when he sees little or no evidence that either the council or the campaign is cutting back on tumour immunology in their own units. By similar reasoning, he also fears for the future of radiobiological research at ICR. With the Medical Research Council about to continue its large Radiobiology Unit under a new director, and with the Cancer Research Campaign committed to its Gray Laboratory in Northwood. Dr Weiss wonders if they will see fit to continue support for radiobiology at ICR, in spite of its good record and close links. with clinical radiotherapeutics. Dr Weiss holds that the institute is better placed to integrate radiobiology with other research activities than other establishments. Peter Newmark

that the MRC could allocate further funds to the ICR because of its divorce from the other two laboratories. But in practice the council has stepped up its commitment to the ICR by just 10 per cent, leaving the funding shared 60:40 with the CRC. This year the council also gave the institute £0.5 million for equipment, but this cannot be guaranteed in the future, says the MRC.

Dr Robin Weiss, ICR director, maintains that the reshuffle has had no immediate effect on the institute. But he is concerned about the future: "With the present arrangement of joint funding, I sometimes worry that we become neither's ultimate responsibility."

In this financial crisis, it would seem logical to predict that the CRC would be loyal to its wholly CRC-funded bodies and that the MRC's responsibility would rest preferentially with its own units. That

leaves the ICR in a rather vulnerable position. For there is no agreement, says Dr Weiss, that the financially healthier CRC should make good where the MRC falters.

Perhaps, though, the concern of the ICR for its future stems from its rough ride through the cuts of 1977. And the MRC is not last to admit that the financial status of the institute is far weaker than that of the Beatson or Patterson laboratories.

And while the CRC and MRC swear allegiance, one message emerges loud and clear, affirming Dr Weiss' concern. The CRC, dependent as it is on finance from the public, can in no way guarantee to act as back-up to the MRC with its government funding under continual threat. It is understandable that the ICR, funded by both bodies, should look nervously towards future government cutbacks.

Susan Douglas

### **NIH** guidelines

Sir - Comments are invited on two proposals for a major revision of the US National Institutes of Health (NIH) guidelines for research involving recombinant DNA molecules. The recombinant DNA Advisory Committee (RAC) has developed a proposed revision and recommended that it be published for comment. This proposal appears in the Federal Register dated 4 December 1981. The major features of this proposal are:

- (1) The guidelines would cease to be mandatory and would become a voluntary code of standard practice. The following requirements would be eliminated: that institutions have an Institutional Biosafety Committee (IBC), that investigators obtain prior approval from the IBC before beginning certain experiments and that investigators obtain prior approval from NIH before beginning certain experiments. The section of the guidelines specifying that noncompliance could lead to loss of NIH funds would also be eliminated.
- (2) Section III of the guidelines giving containment levels would be greatly simplified, and most experiments currently requiring P2 or P3 containment would be recommended at P1.
- (3) The prohibitions section (I-D) of the guidelines would be eliminated, although two of the current prohibitions (I-D-2 and I-D-5) would be retained as admonishments. An alternative proposal appears as item 7 in the Federal Register notice of 7 December 1981. The major features of the proposal are:
- (1) The guidelines would continue to be mandatory for institutions receiving NIH funding. Certain experiments would require prior review by NIH. Certain experiments would require prior review by IBC, and certain experiments would require notice to an IBC simultaneously with initiation of the
- (2) Section III of the guidelines would be reorganized and simplified. All experiments would fall into one of four classes. Physical containment requirements for some classes of experiments would be lowered.
- (3) Three of five current prohibitions (I-D-2, I-D-4, and I-D-5 in the current guidelines) would be listed in a new section that would continue to require RAC review and NIH approval before initiation. Experiments currently falling under prohibition I-D-1 and I-D-6 could proceed after IBC approval.

Copies of the above-mentioned Federal Register notices as well as a summary comparing the current guidelines with the two proposals are available from the NIH Office of Recombinant DNA Activities. Comments on these proposals should be directed to the attention of the Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4A52, Bethesda, Maryland 20205, USA. These proposals and comments on them will be considered by the RAC at its next meeting on 8-9 February 1982.

WILLIAM J. GARTLAND JR

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### Laotian postscript

SIR — The clandestine US war against Laos during the Second Indochina War was for the United States substantially an air war - in terms of environmental impact almost entirely so. Its operational aspects have been well described1. Laos received more than 2.2 million tonnes of air-delivered munitions, fully 31 per cent of the US air war against all Indochina<sup>2</sup>. The massive wide-area saturation bombing from B-52 stratofortresses alone ultimately added up to 800,000 tonnes of bombs, accounting for 36 per cent of all such missions flown during the war throughout Indochina. The air war against Laos was carried out during the years 1965-73; the three peak years were 1969-71, which were almost equally intense and together accounted for 68 per cent of the Laotian total. An estimated 70 per cent of the entire US air assault upon Laos was in southern Laos (the panhandle), carried out primarily against the supply routes into South Viet Nam (the Ho Chi Minh trail). The remaining 30 per cent of the bombing was directed against northern Laos, to a major extent against the Plain of Jars in central Xieng Khouang province.

Herbicidal chemical warfare attacks by the United States during the Second Indochina War were at one time or another directed against all four of the Indochinese nations of the time, overwhelmingly so against South Viet Nam and in only small but unknown amounts against the others. However, the United States has recently released some relevant information regarding Laos<sup>3</sup>. During

the years 1965-69, the United States flew some 100 anti-plant missions (over 400 sorties), with 1966, the peak year, accounting for 76 per cent of them. Altogether 1,600 cubic metres of herbicides were dispensed, mostly (83 per cent) Agent Orange. This Laotian spraying thus represented 2 per cent of the total Indochina effort. The area sprayed was some 67,000 hectares (not counting overlap), 0.2 per cent of the area of Laos. Most by far of this spraying occurred in southern Laos; and most of the spraying was for purposes of forest destruction to expose the Ho Chi Minh trail. A very small but unknown amount of the total spraying (perhaps 3 per cent, the proportion of Agent Blue dispensed) was devoted to crop destruction in the Plain of Jars and possibly elsewhere

The Plain of Jars was especially hard hit during the war in terms of both social and environmental upheaval4 and is recovering extraordinarily slowly<sup>5-8</sup>.

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### To trans, trans, trans-tricyclo [7,3,1,0<sup>5,13</sup>] tridecane

SIR - We are investigating the mechanisms of reactions of organic compounds in solution, in particular the relationship between a compound's reactivity and its molecular shape. We have prepared a number of new compounds, and the most recent of these has an especially pleasing shape and symmetry.

To celebrate this, we thought that a new form of reporting serious scientific results was appropriate, and the sonnet (Elizabethan rather than Petrarchan) seemed worth investigating. A fuller report, not in verse, is to be given today (17 December) at the Scottish meeting of the Perkin Division of the Royal Society of Chemistry at Strathclyde University.

Shall I compare thee to a single form Of cyclohexane locked with bulky group? Or should it be with that bicyclic norm Whose ethane bridge 1,5 doth fix the hoop?

Thy undistorted, perfect, trans-fused rings By force field calculation hold no strain: No rapid rates which angle bending brings As in bicyclo [3,2,1] octane.

Some help thy ground state conformation needs To free an equatorial tosylate, So ring-flip from an all-chair form precedes Reaction from a boat transition state.

And then departs the leaving group when trans Coplanar hydrogens the rate enhance.

Chemistry Department, University of Stirling, UK







H. MASKILL

# NEWS AND VIEWS

# DNA supercoiling by DNA gyrase

from L. Mark Fisher

THE enzyme DNA gyrase is essential for bacterial viability and plays a critical role in DNA replication and repair, transcription and recombination. Since last reviewed in these pages<sup>1</sup>, gyrase has been the subject of intense study and we are now beginning to understand how it carries out its function of catalysing the ATP-dependent introduction of negative supercoils — a coiling of the DNA helix axis itself — into circular duplex DNA.

Supercoiling can be understood in the following way. In linear duplex DNA, the two antiparallel DNA strands form a righthanded helix with about one helical turn for every ten base pairs - the B configuration. If the DNA is in the form of closed circles and has the same helix pitch as in the B configuration it is said to be relaxed. If, however, the two strands of the duplex circle are intertwined fewer times than in the relaxed circle, the DNA minimizes its departure from the energetically favoured B configuration by coiling about its helix axis (see Fig. 1). The degree of this supercoiling depends on the difference between the linking number (the number of times the two DNA strands are intertwined) of the DNA circle and the linking number of the same DNA in the relaxed state. By convention, underwound DNA forms 'negative' supercoils while overwound DNA forms 'positive' supercoils.

The mechanical strain energy stored in negatively supercoiled DNA exerts a persistent torsional stress tending to unwind local regions of the DNA double helix (Fig. 1). Thus, negative supercoiling catalysed by gyrase facilitates processes that entail unwinding of the helix, for example, at replication forks, during transcription and in the DNA strand transfer reactions involved in DNA recombination.

Gyrase belongs to a group of enzymes called DNA topoisomerases that change the linking number of circular DNA. They act by temporarily breaking and then reforming DNA phosphodiester backbone bonds. For all topoisomerases, transient DNA breakage is thought to proceed via the formation of a covalent enzyme–DNA intermediate that conserves the bond energy of the broken DNA phosphodiester bond for later rejoining. Topoisomerases

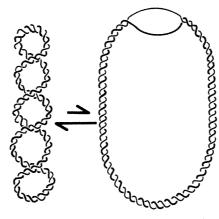


Fig. 1 Underwound circular duplex DNA (shown schematically on the right) forms negative supercoils (left).

are of two types, depending on whether they transiently break one DNA strand (type I) or both strands (type II)<sup>2</sup>. The omega protein of *Escherichia coli* is an archetypal type I enzyme and the eukaryotic nicking-closing enzymes also belong to this class. In contrast, gyrase, phage T4 DNA topoisomerase and some recently discovered eukaryotic topoisomerases are type II enzymes<sup>2</sup>. Gyrase is unique in catalysing DNA supercoiling: all other topoisomerases examined so far catalyse the removal of DNA supercoils, that is, they 'relax' DNA.

Two lines of evidence indicate that gyrase acts by making an enzyme-bridged transient double-strand break in DNA and passing another duplex segment through the break. First, gyrase can tie and untie knots in duplex DNA and can mediate the formation and resolution of catenated (interlocked) duplex DNA circles2-4. Second, both the supercoiling and ATPindependent relaxing activities of gyrase change the linking number of the substrate DNA in steps of two4.5. This result is precisely that expected from passage of one duplex DNA segment through another (Fig. 2). Type I topoisomerases change the linking number of circular DNA in steps of one, consistent with mechanisms involving

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single-strand breakage of DNA5.

How does gyrase catalyse DNA breakage? Information regarding this question has come from studies using the gyrase inhibitor, oxolinic acid. In the presence of the drug, E.coli gyrase forms a complex with DNA that on disruption with a detergent induces double-strand DNA cleavage at specific sites<sup>6,7</sup>. Breakage of complementary DNA strands occurs with a 4 base pair (bp) stagger and results in covalent linkage of the two A subunits of the A2B2 gyrase complex, one to each 53 phosphate end of the break8-12. The A protein-DNA linkage is via an O<sup>4</sup>-phosphotyrosine bond, the same linkage used by some type I topoisomerases in attaching to DNA11,13. Thus, the gyrase A subunits are presumably engaged in breaking and resealing the DNA during catalysis, whereas the B subunits, known to bind ATP, participate somehow in energy transduction. Although the DNA sequences of many gyrase cleavage sites have now been determined, no readily apparent sequence rule governing cleavage has been uncovered8-10

The work of Liu and Wang showed that when gyrase binds to DNA, there is a positive superhelical wrapping of DNA on the outside of the enzyme<sup>14,15</sup>. Thus the gyrase-DNA complex resembles in part the nucleosome formed between histones and DNA, in which the DNA is known to be on the outside of the histones, although wrapped with a negative superhelical sense. Recently, detailed structural information about the gyrase-DNA complex has been obtained by several groups using nuclease protection methods<sup>9,10,16</sup>. These studies made use of specific gyrase binding sites on DNA, which were identified using the oxolinic acid cleavage reaction, or in the case of Micrococcus luteus gyrase, by a DNA binding assay. Gyrase bound at these sites protects 100-140 bp of DNA against DNase I, contained within the 143 bp of DNA protected against staphylococcal nuclease<sup>15, 17</sup>. Protection is observed both in the presence and absence of oxolinic acid. A 40 bp region encompassing the oxolinic acid cleavage site is most strongly protected. Assuming that gyrase cleavage sites are sites of transient DNA breakage

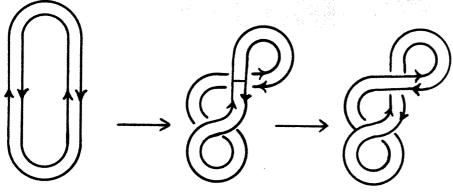


Fig. 2 Passage of one duplex DNA segment through another changes the linking number of circular duplex DNA in steps of two. A circular duplex DNA of unique linking number (left) is folded (centre) bringing together two different segments of DNA helix. Breakage of both strands of one helical segment, passage of the second helical segement throught the break followed by resealing generates a DNA molecule (right) differing by two turns from the starting DNA. Thus, strand passage has changed the linking number of the DNA by two.

during catalysis, then this 40 bp segment presumably forms part of the gate through which the second DNA duplex is passed. Protected DNA flanking this region contains sites of enhanced DNase I sensitivity spaced on average 10 or 11 bp apart, in a pattern similar to that observed for DNase I digestion of nucleosomal DNA. This result is diagnostic of DNA adsorbed to a surface and indicates that DNA flanking the cleavage site is wrapped around the outside of gyrase.

Several related models incorporating double-strand DNA breakage have been advanced to explain how gyrase supercoils DNA. All of the them embrace the following basic scheme. First, gyrase binds to DNA resulting in a positive wrapping of DNA around the enzyme and the formation of a compensating negative turn elsewhere in the DNA. ATP binding allows transient DNA breakage and passage of a second DNA segment through the break into the enzyme complex. The break is sealed and then ATP hydrolysis promotes release of the translocated DNA segment ready for another cycle. This sequence of events has been deduced in part from the finding that the non-hydrolysable ATP analogue ( $\beta$ ,  $\gamma$ -imido)ATP promotes only a single cycle of DNA translocation<sup>18</sup>. Wang et al. suggest that the positive superhelical wrapping of DNA on gyrase is retained during catalysis and that the translocated DNA segment can be anywhere in the nearby DNA19. Mizuuchi et al.4 and

Morrison and €ozzarelli<sup>16</sup> propose that the translocated DNA segment is directly contiguous with the wrapped DNA. Strand passage then results in conversion of the wrapped positive DNA turn on gyrase directly into a negative turn, thereby accounting for the directionality of the supercoiling reaction. It is unknown how the DNA duplex passed into the gyrase-DNA complex during catalysis is subsequently released. The DNA could be shuttled along an interface between gyrase subunits ultimately emerging from the gyrase complex at a location different from its entry point<sup>4, 19</sup>. Alternatively, a single entrance/exit port could be used, in which case, release of the passed DNA would require prior unwrapping of the resealed cleavage site16. These problems await clarification.

Type II enzymes have recently been isolated from eukaryotic cells2, 20, 21. Like T4 DNA topoisomerase, they do not supercoil DNA but act as ATP-requiring DNA relaxing enzymes. Intriguingly, they use ATP even though the removal of DNA supercoils is favoured thermodynamically. The biological functions of eukaryotic type II (and type I) topoisomerases remain to be elucidated.

### **Site-specific recombination**

from David Sherratt, Avril Arthur and Paul Dyson

GENETIC RECOMBINATION can conveniently be divided into two types distinguished by the extent of the DNA homology in the region of recombination. Homologous (or generalized) recombination occurs efficiently only between regions of DNA having at least several hundred homologous base pairs whereas non-homologous (or illegitimate) recombination occurs at regions of little or no homology. At least in Escherichia coli these recombination types are also distinguishable by the enzymes required: in vivo and in vitro experiments show that the RecA protein is directly involved in homologous recombination, whereas nonhomologous recombination is RecA independent and is often promoted by proteins whose genes are adjacent to the recombination site. Non-homologous recombination can be accompanied by DNA replication, for example in genetic transposition, or can simply be a conservative reciprocal breakage and reunion of DNA strands as first proposed by Campbell for bacteriophage  $\lambda$  site-specific recombination in 19622,3.

Several recent publications show that site-specific recombination is utilized in prokaryotes for a variety of functions, and though different systems show some similarities, their underlying molecular mechanisms may differ to allow efficient execution of their normal biological functions. The different possible outcomes of reciprocal recombination are schematically outlined in Fig.1, which also gives some indication of the activities and preferred directionality of the systems to be described here.

Bacteriophage  $\lambda$  integration and excision is the archetype of site-specific recombination and Campbell's model has been elaborated and substantiated by a wealth of elegant biochemical and genetic data<sup>4-6</sup>. Integration of  $\lambda$  in vivo requires  $\lambda$ int protein and normally occurs between attP, a region of about 240 base pairs (bp) on  $\lambda$ , and attB, a region of some 25 bp on the bacterial chromosome, generating two hybrid sites attL and attR. Though attP and attB are non-identical, they share a 15 bp homologous 'core' sequence within which recombination occurs. The reverse recombination reaction, between attL and attR, which leads to  $\lambda$  excision in vivo, requires both int protein and a second  $\lambda$ protein specified by the xis gene. In vitro, int promoted recombination occurs on negatively supercoiled DNA molecules containing both attP and attB, with little or no loss of supercoiling, no net DNA synthesis and no need for an exogenous energy source such as ATP. However, The authors are in the Genetics Department,

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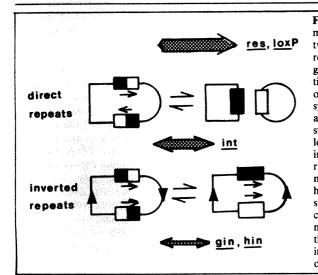


Fig.1 Consequences of intramolecular recombination between direct and inverted repeats. The hatched arrows give some indication of the activities and preferred directions of the different recombination systems. Whereas int protein acts on all combinations of substrates, Tn3/yô res and P1 lox appear primarily to act intramolecularly on directly repeated substrates. The normal biological function of hin and gin is to act on inverted substrates. Note that if any recombination sites are symmetrical, that is, palindromic, then repeats of them would be in both direct and inverted orientation.

there is a requirement for a host-protein complex, though int-protein alone has topoisomerase activity, that is, it can mediate breakage-reunion reactions.

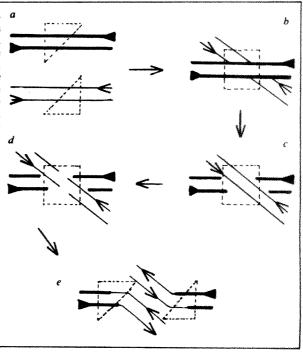
In contrast to λ-mediated site-specific recombination which can occur both intermolecularly (during normal integration) and intramolecularly (during excision), some other site-specific recombination systems, that have very different biological functions, usually operate intramolecularly at sites of fixed orientation. For example, in bacteriophage Mu, the expression of either of two different host ranges is specified by the orientation of an invertible segment of DNA bounded by a short inverted repeat. An adjacent gene, gin, specifies a recombination enzyme that acts at these repeats to mediate the inversion7. A closely related (and interchangeable) enzyme (hin protein) can similarly invert a l kilobase (kb) region of the Salmonella typhimurium chromosome by recombination across a 14 bp repeat to determine which of two flagellar types is synthesized?. In contrast to these two examples, where site-specific recombination acts to invert a sequence (exerting a 'flip-flop' phenotypic control), two other systems preferentially act on molecules containing directly repeated copies of the recombination site (Fig.1). Bacteriophage P1 exists as a plasmid in its lysogenic state and has a site-specific recombination enzyme which rapidly converts any dimeric plasmid molecules (generated during replication or homologous recombination) to monomers, by intramolecular recombination at a specific site, loxP8. It is postulated that such recombination may be important for stable maintenance of P1 as a plasmid, since its copy number control is so stringent that P1 replication is inhibited once there are two P1 replication origins in a cell. A single P1 dimer cannot be partitioned to both daughter cells.

Transposons of the Tn3 family also specify site-specific recombination systems that act most efficiently on directly repeated copies of the recombination site (res) within the same molecule<sup>9-11</sup>. Such molecules are generated in the first step of

intermolecular transposition of these transposons and are rapidly resolved to the normal transposition end products by the site-specific recombination. Two recent papers by Reed and Grindley<sup>12,13</sup> characterize the essentials of the reaction in vitro for one Tn3 family member, yô. The only requirements are the recombination protein (resolvase), Mg2+ and a negatively supercoiled circular DNA molecule containing two directly repeated copies of res. Although relatively large amounts of resolvase are required, the substrate is rapidly converted to the in vitro reaction products, two interlinked circles. In the absence of Mg2+, a limited reaction occurs in which a resolvase-DNA complex accumulates. Removal of the protein from the complex with protease leads to the appearance of linear DNA fragments that are the result of double-strand breaks at each of the res sites. The breaks are staggered, having two nucleotide 3' extensions, and have remnants of the resolvase bound to the 5' ends. Formation of such fragments depends on two res sites being present in the same DNA molecule, indicating that the breakage reaction can only be initiated on formation of a complex of resolvase bound to two res sites. If these resolvase-DNA complexes are subsequently incubated with Mg2+, then some of them are converted to the normal in vitro products, supporting the idea that the staggered breaks are intermediates in the recombination. It therefore seems that yo resolvase, like known topoisomerases, can conserve polynucleotide chain phosphodiester bond energy generated during strand breakage and use it for subsequent strand rejoining. Although similar intermediates have not been reported in the  $\lambda$ int-promoted reaction, experiments have shown that five or seven nucleotide staggered breaks with 5' extensions occur during such recombination5.

Nash and co-workers have suggested that during & recombination the two homologous 'cores' come together to form a four-stranded structure stabilized by intermolecular hydrogen bonds, a structure originally proposed by McGavin<sup>14</sup>. The int protein-promoted nicking of a single strand at the same position on each duplex could allow rotation around the uncut strands to bring broken ends adjacent to their recombinant partners, where ligation occurs. Subsequent nicking, rotation and ligation of the other two strands would produce recombinant DNA6. Though such a model is consistent with the type I topoisomerase properties of int protein, strand rotation appears not to be an inherent property of typical topoisomerases15. Reed's and Grindley's observations that γδ resolvase can generate a double-strand cleavage could mean that this enzyme acts like a type II topoisomerase, the recombination occurring as one cleaved res site undergoes strand passage through the

Fig.2 A model for res recombination. (a) Two regions of DNA each containing at least a dimer of resolvase bound to res sites, (b) protein interactions bring the two res sites together and now (c) double-strand cleavage of one of the helices occurs and a double-strand passage event is initiated, as during type II topoisomerase action 15. Strand separation and rotation are prevented by bound enzyme. During the strand passage, the second cleavage occurs (d) and is immediately followed by ligation to produce recombinant DNA. Though interlinking of helices to give catenanes will arise from accidental interwinding of DNA segments that will eventually reside in different recombinant products6, it could occur during strand exchange at the



second (Fig.2). Such a mechanism would implicate resolvase protein rather than four-stranded DNA both in initial synapsis and in the maintenance of superhelicity during recombination.

As more information becomes available it is becoming clear that despite similarities in these recombination systems, they have evolved diverse biological roles. For example, whereas the \(\lambda\) int, Tn3/y\(\delta\) res and P1 lox systems may need to act very rapidly, inversion in the gin and hin systems occurs relatively rarely. This is to be expected, since there would be little sense in rapid switching from one host range or flagellar type to another. The relative efficiencies of these systems may reflect both the activities and amounts of the recombination enzymes and the nature and position of the normal recombination sites. Because the energy states of the reaction substrates and products ought to be very similar, the presence of a functional site-specific recombination system should generate an equilibrium mixture of similar quantities of reactants and products. Though this appears to be the case for the inversion in the 'flip-flop' recombination systems, the other systems have evolved ways of apparently shifting this equilibrium. If  $\lambda$  excision were simply a reversal of integration, then establishment of stable lysogeny would be difficult or impossible. The asymmetric  $\lambda$ recombination sites (attP and attB) generate different integration and excision substrates, which in turn allow the additional xis protein to be required for excision. At first sight, the Tn3 and yô sitespecific recombination reactions appear as if they should be readily reversible: in a molecule containing two directly repeated transposons there is at least 5 kb of homology surrounding the res sites, yet though such a molecule is an excellent recombination substrate, the reverse reaction to join two separate molecules through res recombination appears very infrequent. Perhaps even more surprising, res recombination in vivo or in vitro acts poorly on inverted copies of res in the same molecule. In contrast, λ int protein can act on attP and attB in all configurations (Fig.1). We presume that topological considerations may determine the very different efficiencies with which res recombination takes place on the different substrates. DNA topology is likely to be important in control and determining specificity in many biological systems.

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# A plasma wake for Mars' satellite Deimos

from Alan Johnstone

SUCH diverse topics in space physics as the formation of the Earth's magnetosphere and the electrical charging of spacecraft can be gathered together under one heading — the interaction between a plasma and a moving body. The physical processes involved are quite varied and depend on the size of the body and its physical characteristics.

Although some experiments have been carried out in the laboratory, the conditions in space cannot be modelled accurately. Empirical knowledge of the interactions in space is therefore limited to observations of naturally occurring cases where the parameters cannot be varied in controlled conditions. Fortunately, the exploration of the Solar System in the last decade has revealed an unexpectedly rich variety of examples which cannot even be listed in a short article. Now, yet another one has been discovered. One of the martian satellites, Deimos, which is only 15 km across its largest dimension, is believed to create a disturbance in the solar wind which is 5,000 km across, 15,000 km downstream from the object (Bogdanov J. geophys. Res. 86, 6926; 1981).

The solar wind is an extremely tenuous gas of positively and negatively charged particles [10 per cm<sup>3</sup>] flowing outwards from the Sun at hypersonic speeds (400 km s<sup>-1</sup>). The Russian spacecraft Mars 5, in orbit around Mars, found a region where the plasma density was lower than in the solar wind, the velocity was slower and the temperature higher at a time when the spacecraft was well upstream from any interaction with the planet itself. Another spacecraft, Mars 4, one million kilometres upstream from Mars at the same time, and which normally measured the same plasma parameters 20 minutes earlier than Mars 5, did not encounter a similarly disturbed region nor did it observe a variation in solar wind conditions which could have caused the martian bow shock to have formed in an unusual position upstream. Throughout its passage through the disturbed plasma Mars 5 continued to measure the same strength and direction for the magnetic field as it had in the solar wind.

When the solar wind encounters a solid body which absorbs the particles that strike it, like the Moon, then it simply leaves a wake little larger than its diameter extending tens of diameters downstream. If Deimos created the effect observed by Mars 5 it must interact more strongly than this. If the body deflects the particles, and if there is a strong enough force between the particles to organize their motion in a collective way, then the solar wind will flow around the body with a fluid-like motion, thereby creating a much larger disturbance downstream. Since the average distance between collisions is greater than the distance from the Sun to the Earth, it cannot be a fluid of the type we know in everyday life. In fact, the magnetic field frozen in the plasma organizes the motion and is strong enough when the gyroradius of the ions is small compared with the scale length of variations in the flow. The solar wind can be deflected by a magnetic field but, since the gyroradius in the solar wind is about 100 km, Deimos would need a relatively large dipole moment to create an obstacle capable of deflecting the solar wind as a fluid. If Mars 5 had crossed a magnetic tail from Deimos then the magnetic field should have been aligned antiparallel to the solar direction. It was actually at an angle of 70° to this direction, so it is unlikely that the interaction is magnetic.

The flow can also be deflected by an atmosphere, as at Venus. There the upper atmosphere is ionized by sunlight and induced currents deflect the solar wind. The gravitational field of Deimos is far too weak to hold a permanent atmosphere but if there is a continuous outgassing from the surface then the density of the outwardflowing gas could perhaps be sufficient. The molecules do not interact with the solar wind until they have been ionized either by sunlight or by charge exchange. The interaction suggested by Bogdanov is one where the thermal pressure of the electrons created by photoionization is sufficient to balance the solar wind pressure. In order to obtain a fluid-like interaction the surface of pressure balance must be placed at least an ion gyroradius from the surface. The outgassing rate required to achieve this would result in the loss of one-third the mass of Deimos in a billion years. The outgassing could therefore be sustained for a period comparable with its lifetime.

Bogdanov compares this process with the interaction between a comet and the solar wind. Water, carbon dioxide and other volatile compounds sublime from the surface of the comet's nucleus as it is heated during its passage near the Sun. As this gas flows outwards from the nucleus it creates most of the familiar visible features associated with a comet. The outgassing rate from Deimos, required by Bogdanov's theory, is only one-millionth that of a large

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comet near perihelion so Deimos should not produce a large visible coma.

While Bogdanov's mechanism may be important near the body, the outgassing should be important over larger distances and near comets. When the neutral molecules are ionized by sunlight they are accelerated by the electric and magnetic fields up to the solar wind speed. This addition of mass to the solar wind flow requires a reduction in speed to conserve momentum. These implanted ions follow cycloidal paths around the magnetic field direction and would not be detected by particle detectors aimed at the solar wind. The radius of the wake is roughly equal to the average distance travelled by a gas molecule before ionization. This mechanism should produce a larger wake at a lower outgassing rate.

Clearly the observations make Deimos a more interesting body but whether Deimos is the cause of the disturbance and, if so, which mechanism is responsible can only be determined by more measurements from spacecraft.

# Thermal instability of solar loop structures

from Robert Rosner

THE past decade has seen a fundamental revision of our conception of the spatial structure of the multi-million degree plasma (corona) surrounding the Sun<sup>1,2</sup>. The change involves recognition of the dominant role of the magnetic fields, which pass outwards from the solar interior through the visible layers of the solar surface, in structuring the overlying coronal atmosphere into regions topologically 'closed' and 'open' to the interplanetary medium.

The consequences for theoretical models of the solar corona have been quickly realized, primarily in calculations based on simple hydrostatic models3,4 involving magnetic plasma confinement. In the past few years, the existence and stability of these equilibrium solutions to the hydrostatic equations of motion have been extensively studied<sup>5-8</sup>. The extreme nonlinearities of the problem make the temporal behaviour of the confined solar atmosphere very difficult to predict theoretically. Especially troubling has been a discrepancy between theory and observation: most theoreticians believed that unless thermal conduction from the coronal loop down to the solar surface was 'large', instability was inevitable (so that the loop atmosphere was then unlikely to be in a state approximating hydrostatic conditions8), but X-ray observations of the Sun showed that the confined multimillion degree solar corona had little difficulty in maintaining plasma conditions often observationally indistinguishable from a quiescent, stationary state<sup>2</sup>. The extent to which the predictions based on simple equilibrium loop models match the data is quite remarkable9. The disparity is of great general interest because related problems of thermal and magnetohydrodynamic stability have arisen in laboratory plasma studies and interstellar medium and extragalactic plasma astrophysics; the solar

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corona thus provides a 'laboratory' in which plasma stability can be put to test. In a recent issue of Nature Craig and McClymont<sup>10</sup> show how a large part of this difficulty can be resolved by more realistic modelling of the low-temperature boundary layer separating the coronal loops from the visible solar surface.

The early work on stability focused on two distinct issues: assuming isobaric perturbations, first, are the equilibrium solutions stable if the base temperature is held fixed; second, are the equilibrium solutions stable if the base conductive flux is held fixed. The first case (A) would seem to be appropriate if the base is located sufficiently deep in the atmosphere so that coronal (or transition region) pertubations do not disturb local energy balance. The second case (B) is more complex: the conductive flux may remain fixed either at a fixed spatial location (with varying base temperature; case B.1), or for a fixed temperature (with varying base location; case B.2); the latter case is appropriate if the base of the loop is chosen to lie in regions where radiative processes can control the temperature rise (see ref. 11), so that the base takes on the character of a free surface whose spatial location may vary. It is not obvious which of these three alternatives is in fact followed by the Sun's atmosphere.

Results for case A (which assumes unvarying conditions in the underlying chromospheric plasma) showed instability was inevitable if the base thermal conductive flux was very small ('thermally isolated' case), but with growth rates sensitively dependent on the ratio of base to peak temperature<sup>5-7</sup>. The basic physics of the instability involves the strong destabilizing influence of radiative energy losses (which decrease with increasing temperature for coronal plasma temperatures), and the stabilizing effect of thermal conduction, much as discussed in the classic paper by G. B. Field<sup>12</sup>, but recast in the context of the strong inhomogeneities found in the solar

atmosphere (so that effects of boundaries, for example, must be taken into account).

In contrast, it has been argued that for case B.1, instability is inevitable, but that for case B.2 the stability of hydrostatic equilibrium solutions depends both on the ability of the loop base to adjust to coronal (or transition region) perturbations and on the response of the local heating process to imposed perturbations6.

Two very distinct approaches have been followed in trying to resolve this impasse. Recently, several groups have developed numerical time-dependent hydrodynamic codes which are capable of following the evolution of solar coronal structures; these studies follow the evolution of perturbed equilibrium models, and appear to conflict with much previous analytical work: imitating nature, the loop simulations show great robustness against perturbation<sup>13-15</sup>. The work of Craig and McClymont<sup>10</sup> avoids the necessity for such 'brute force' calculations by asking a simpler (yet crucial) question: can the earlier analytical results predicting strong instability for fixed conductive flux conditions be maintained if one takes into account the rapid temperature variation along magnetic field lines at the photospheric base of the coronal loop structures; specifically, can a change in the (de)-stabilizing properties of the radiative energy losses at low temperatures significantly affect loop stability? This question's relevance is clear from the earlier results, which typically showed that the denser and cooler portions of the loop atmosphere tended to control the instability time scale5.

Starting with a realistic radiative energy loss function (in particular, at low temperatures), Craig and McClymont proceed to calculate the linear growth rate of the most unstable mode for a model with a fixed, vanishing base thermal conductive flux (case B.1 above), as a function of the amount of low-temperature material included at the base of the model atmosphere. The basic result is that, in typical solar conditions, the instability time

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scales for the unstable modes far exceed typical observed lifetimes (viz., days) of the loop structures in question; remarkably little low-temperature material is, in fact, required to quench the instability. Theory can thus now be fully reconciled with observation: if proper account is taken of the stabilizing influence of the boundary layer separating the solar corona from the underlying (visible) photosphere, analytical normal mode analysis does predict stability for time scales of the order of the observed coronal structure lifetime:

the error of previous calculations — and the source of the apparent discrepancy with observations — was simply the neglect of this stabilizing factor.

From both the observational and the theoretical perspectives, it thus now appears that the gross properties of the confined solar corona can be understood by considering simple hydrostatic equilibrium models, although the reasons for this are quite complex and, in some cases, are still not well understood9. However, when one looks at the corona in

some detail the simplicity disappears, and it is apparent that hydrostatic models are seriously inadequate. Observations of intensity fluctuations, of persistent up and down flows, of relatively cool matter residing at coronal heights, of apparent spatial mingling of hot plasmas at quite different temperatures, all call for more sophisticated modelling. Analytical methods seem inadequate for such modelling; the new plasma simulations have thus appeared at an opportune time.

## Hybridoma technology identifies protective malaria antigens

from F.E.G. Cox

VARIOUS partially successful attempts have been made to immunize experimental animals against malaria but preparations of the most easily obtained antigens, those derived from the forms of the parasite in the blood, have been relatively crude and poorly characterized. The use of monoclonal antibodies has now enabled scientists at the Wellcome Research laboratories to identify specific protective antigens and to use them to immunize mice against a virulent form of malaria1.

A vaccine against malaria is one of the prime needs in tropical medicine and in a variety of models in laboratory animals vaccines have been prepared from sporozoites, the infective stages injected by the mosquito; schizonts, the dividing forms in the red blood cells; and merozoites, the products of this division that invade fresh cells. Monoclonal antibodies have been used to identify an antigen with a molecular weight of 44,000 that completely envelops the sporozoite and induces immunity to the rodent malaria parasite Plasmodium berghei<sup>2</sup>. This antigen is specific to the sporozoite and seems to be a differentiation antigen that appears while the sporozoites are in the salivary glands of the mosquito and disappears soon after they have been injected by the mosquito and have entered the liver cells where they first phase undergo their multiplication3.

Although a sporozoite vaccine is a logical and feasible possibility and would prevent the parasite from developing in the liver and thence the blood, a number of workers feel that should a single sporozoite escape the immune response, infection of the red blood cells would eventually ensue and therefore a vaccine should be based on the blood stages. The most promising candidate is the merozoite, and injections of merozoite antigens combined with adjuvants have been shown to protect monkeys against several species of malaria parasite4.

A monoclonal antibody against merozoite antigens of the rodent malaria parasite, Plasmodium yoelii, is known to confer protection on passive transfer5 and it has now been used to identify the antigen involved1. The latter has a molecular weight of 235,000, is associated with an internal organelle of the merozoite and is specific to the merozoite. When mice were immunized with this antigen in Freund's adjuvant, they responded with high levels of merozoite-specific antibody and were protected from death following challenge, although they experienced relatively high parasitaemias lasting about 16 days. A second monoclonal antibody known to react with schizonts, but which was not protective on passive transfer<sup>5</sup>, was used to identify a second protective antigen. This is a surface antigen with a molecular weight of 230,000 which is present early in the maturation of the schizont and occurs on both schizonts and merozoites. Mice immunized with this antigen produced high titres of antibody against schizonts and were very resistant to challenge, experiencing only a mild infection lasting about 9 days. The significance of these results is that monoclonal antibodies can be used to identify protective antigens, but it is also interesting that the best protective immunity was produced in response to the antigen whose corresponding monoclonal antibody failed to provide passive protection.

What exactly is the nature of these antigens? Using similar virulent and avirulent forms of P. yoelii, Taylor et al.6 also have produced a range of monoclonal antibodies to sporozoites, schizonts and merozoites. Their antibody to merozoites was specific to merozoites but crossreacted with those of several species, was associated with an internal organelle possibly involved with penetration into the red blood cell but was not protective. Epstein et al.7, using Plasmodium knowlesi in monkeys, have obtained three hybridoma lines that produce monoclonal antibodies against merozoites. All three agglutinate merozoites and two block red cell invasion. The antigen involved has a

molecular weight of 250,000 (or 240,000 if saponin lysis of infected cells is used) and cross-reacts with various strains of P. knowlesi. A fourth group of workers (Perrin et al. 8) have also raised monoclonal antibodies to various stages including merozoites, this time to the human parasite, Plasmodium falciparum, in the mouse, a non-susceptible host. The merozoite antigens involved were surface polypeptides with low molecular weights, 36,000 and 96,000, and antibodies against these inhibited the development of P. falciparum in vitro. Antigens with low molecular weights like these must be viewed with caution, because Holder and Freeman also detected low molecular weight polypeptides associated with schizonts and concluded that they were proteolytic fragments of the 230,000 molecular weight antigen1.

Thus a number of potentially protective antigens on the merozoite have already been identified using monoclonal antibodies. Some are surface antigens and some are internal, some cross-react with other stages or the merozoites of other species and there is also variation in molecular weight. It is likely, therefore, that a number of antigens are involved in the immune response to the merozoite alone and these may operate sequentially, concurrently or alternatively. Antigens associated with schizonts1 and sporozoites2,3 have also been identified using monoclonal antibodies. It is going to be a long time before anyone identifies the most important protective antigens in a malaria parasite but in the meantime there will be a quantum jump from the use of crude homogenates as vaccines to cocktails of characterized polypeptides and these are much more likely to be acceptable in human medicine than anything in experi- $\Box$ mental use at the present time.

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# Which direction for ocean drilling?

from J.R. Cann

A recent conference\* on ocean drilling (COSOD) enabled some one hundred and fifty geologists and geophysicists — including both those involved in the field as well as sceptics from other areas — to take a cool look at the long-term future of scientific drilling of the ocean floor. Of particular interest was the debate over whether the drillship Glomar Challenger should continue to be used or whether it should be replaced, at considerable expense, by conversion of the much larger Glomar Explorer.

Ocean drilling has been conducted since 1968 from the special dynamically positioned drillship Glomar Challenger, originally entirely USA funded, but expanded in the mid 1970s to include participation by the UK, France, Germany, Japan and the USSR. Currently the project costs \$21 million per year of which \$14 million is required to run the ship. The non-US subscription runs at \$2 million per year per partner, on top of which is the spending required to support the experiments connected with drilling.

Following a similar conference in 1976, plans were initiated to convert the six times larger Glomar Explorer from its original deep lifting role to a vessel for deep ocean margin drilling, carrying all the safety equipment necessary for drilling holes 6 km deep in water 3 km deep. Although at first it seemed that the Challenger and Explorer programmes might develop in parallel, progressively revised cost estimates showed that this was unlikely.

Scientific returns from Challenger increased in quality, especially with the development of the hydraulic piston corer that recovers almost undisturbed sediments from as deep as 200 m below the ocean floor, but financial pressures became greater. The planned Explorer drilling was too costly for the US Government to undertake alone, and under President Carter the joint government/oil company Ocean Margin Drilling Program was evolved. Eventually, after an initiative from the NSF to merge the two programmes, the oil companies withdrew from the Explorer program on 6 October this year. A number of detailed geophysical compilations for the areas selected for deep drilling (to be available next year) were carried out as well as various feasibility studies for the engineering involved. A Lockhead-Sedco venture is currently studying how best Explorer could be converted - without the expensive and complex safety systems necessary for really deep penetration in deep water.

\*The 'Conference on scientific ocean drilling' (COSOD) was held in Austin, Texas on 16-18 November, 1981. The steering committee is preparing a report which will be available in the spring. COSOD, which was originally conceived as coordinating the *Challenger*- and *Explorer*-based drilling programmes, was left with two principal tasks, both of greatest importance. One was to examine the scientific justification for drilling beyond 1983, and the other to consider, if drilling was to continue, whether using *Explorer* or *Challenger* would be best.

A. Shinn, newly appointed director of the Office of Scientific Ocean Drilling at NSF, set the framework from the NSF point of view. He pointed out four options: (1) to terminate all scientific drilling at the end of 1983, when *Challenger* finishes her current phase of operations; (2) to extend the *Challenger* program for up to five more years; (3) to convert *Explorer* to a *Challenger*-like ship and use her instead of *Challenger*; and (4) to convert *Explorer* to a ship fully equipped for deep penetration in deep water as envisaged by the Ocean Margin Drilling Program.

Option (1) he presumed, correctly, would be rejected by the conference, and option(4) he regarded as too expensive to support, leaving options (2) and (3). He indicated that running costs for *Explorer* would be similar to or somewhat more than those of *Challenger*, but that conversion of

Hairstar from the Taimur Coast. From Nature 25, 22 December, 181 (1881).

Explorer would put a halt to drilling (so that conversion could be funded) for about 18 months. He asked the conference to help decide between the two options, by assessing the ability of Explorer to handle larger amounts of drilling materials, to drill in high latitudes (especially in the Southern Ocean), to carry more scientists and to increase in drilling capability through a longer life, compared with the known and continuously available capability of Challenger.

A series of scientific reports and workshop discussions followed, including presentations from planning groups associated with both the present Challenger drilling and the Ocean Margin Drilling Program originally planned for Explorer.

On the final day the chairmen of the working groups presented their proposals to the reassembled conference, together with priorities for drilling. There were far too many proposals for excellent science than could be accommodated by one drilling ship. Among the highest priority targets presented, 'natural laboratories' was one watch word. These would consist of groups of holes in the ocean crust to study hydrothermal circulation, crustal construction and tectonics, which would act not just as sources of rocks for examination, but also as sites for in situ measurements and experiments, both in the short term and as long-term observatories. Work of this kind has already started, and shows great promise. Some holes would be sited on young, newly created crust where water at 350°C is seen to bring out sulphide ores from within the crust, and technical problems of drilling on bare, sediment-free rock, as well as at these very high temperatures, must be solved.

Testing the Vail Curve was another priority. This curve shows sea level in the geological past and was constructed by Peter Vail and his associates at EXON on the basis of seismic reflection profiles and drilling in shelf areas. Various aspects can best be tested outside traditional oil-producing areas, including the magnitude, suddenness and synchronism of the changes, and a number of such tests were proposed. If the Vail Curve can be made entirely reliable, then the consequences for understanding the geology of sediments would be great indeed.

Island arc regions, near the chains of volcanoes that border the Pacific and some other parts of the oceans, are places where plates are being subducted back into the Earth's mantle. They are still poorly understood, and are clearly of great importance in understanding global geology. Many kinds of targets were identified here, of which the most graphic is the drilling through the pile of sediments on the inner wall of ocean trenches into the descending plate of oceanic material,

J.R. Cann is Professor of Geology at the University of Newcastle upon Tyne. where experiments could be performed. If much of the oceanic sediment is swept down into the mantle here, as seems increasingly likely, this could have profound effects on the chemical balance of the Earth's interior

Palaeoclimates are inextricably linked with past oceanic circulation. In particular, the Antarctic regions carry the full record of glaciation and deglaciation, as well as an amplified record of pre-glacial climates. This is a prime target for any further drilling, but others too were identified in this area, such as the history of the vertical temperature and oxygenation structure of the deep oceans, and the nature of the great Pacific Ocean of 200 Myr ago. Only small fragments of this ocean crust remain, and these are buried deep beneath later sediments. However the Pacific then was even larger than today, and was essentially the only ocean, lying opposite the supercontinent of Pangaea.

Finally there is the problem of the origin of split continental margins, such as those bordering the Atlantic. Since these areas are closely associated with petroleum generation and might contain deep-water petroleum deposits, they are clearly worth drilling. To understand them you need to drill where oil companies will not — on margins where sediments are thin and the history is close to the surface. During last summer great advances were made in our understanding of these areas by *Challenger* drilling around the Bay of Biscay and Rockall Bank, and this work is to be extended.

Which would be best for all this drilling, Explorer or Challenger? The tools and techniques people presented a report which essentially said that you get what you pay for. Challenger could be upgraded in many ways at a cost of \$7.5 million, although she could not apparently be fully strengthened for work in ice. Explorer could be converted for drilling at a greater cost, but would have greater capabilities, especially if extra money was available. Some of the scientific targets, such as the bare rock drilling, would require extensive engineering development, while others could only be reached using a fully converted Explorer. An informal vote was called for among the scientists present to see the extent of the support for Explorer or Challenger, and a substantial majority felt that the potential of Explorer outweighed the know abilities (and restrictions) of Challenger.

The general view of COSOD was that oceanic drilling should be regarded as integral a part of earth science as radiotelescopes are of astronomy. Instead of the present situation, where continuation of drilling has to be fought for every two years, there should be a general expectation, as with telescopes, that funding will continue and that the great contributions made to earth science by drilling will continue at the same or higher levels over at least the next decade.

### Life seen from a medieval latrine

from Peter D. Moore

IF, as it is said, one can tell much about man's way of life from the contents of his garbage can, then one may indeed be able to tell even more from the contents of his latrine. It is perhaps surprising, then, that such depositories of environmental information have been rather neglected by archaeologists, despite their longestablished affinity for middens. There are exceptions, of course, such as the delvings of Pike and Biddle (Antiquity 40, 293; 1966) into the sewerage system of medieval Winchester in search of human gut parasites and Dennel's (Econ. Bot. 24, 151; 1970) analysis of plant macroscopic remains from a sewer in medieval Plymouth. In the New World less is known but nevertheless. Schoewetter (Archaeology of the Mammoth Cave Area, Academic Press, 1974) has been able to analyse the contents of sub-fossil human faeces in Upper Salts Cave, Arizona.

A golden opportunity to develop an interdisciplinary approach to the study of long-abandoned lavatories arose in Worcester in 1975 when the clearance of an area of housing for the development of a new road revealed the existence of the lower part of a medieval barrel latrine filled with organic material. The find was excavated by Greig and its contents analysed for beetles (Osborne), plant macrofossils and pollen parasites (Greigh), bones (Jones) and cloth (Crowfoot and Raphael). It was radiocarbon-dated to the mid fifteenth century and the work has now been reported by Greig (J. archaeol. Sci. 8, 265; 1981).

The beetle fauna was dominated by Tipnus unicolor and Mycetaea hirta, which Osborne considers to be characteristic species of the cesspit habitat. He comments that the scarcity of these species today contrasts strongly with their abundance in medieval times, no doubt a consequence of such developments as the water closet. Of the other 36 beetle taxa recorded, an intriguing find is Anobium punctatum, the furniture beetle or woodworm. It may be that this hapless insect inhabited the (presumed) wooden seat above the latrine and that it died on emergence, but Osborne considers this taxon to be something of a coleopteran 'background noise' in a variety of deposits and therefore of little significance. Other beetles are characteristic of stored grain, or vegetables, which suggests an admixture of kitchen refuse in the barrel. It is possible

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that such material could have been used for hygienic purposes together with the fragments of cloth and moss (tentatively identified as *Thuidium*) also found in the barrel.

Interpretation of the wealth of plant material found in the latrine is complicated by this input of extraneous vegetable matter. Undoubtedly some of the fruits and seeds found have passed through the human gut, such as Robus fruticosus (bramble - an unlikely candidate for hygienic uses), Fragaria (strawberry), Vitis (grape) and Ficus (fig). The figs must surely have been imported but it is possible that the grapes were locally grown. Many fruits and seeds were weeds of cultivation, or hedgerow species which could have found their way into collections of straw, indeed in the case of some of these, such as fennel (Foeniculum vulgare), coriander (Coriandrum sativum) and carrot (Dancus carota), it is difficult to be sure whether their origins are cultivated or casual. Certainly some of the seeds must be derived from the contaminant weeds of medieval crops, such as the corn cockle (Agrostemma githago), all species which have declined drastically in the present century with the advent of herbicides and whose demise is greatly mourned by the pestiphile lobby among conservationists.

Anyone who reads old herbals will be aware of the degree to which our forefathers were obsessed with the activities of their alimentary systems. It is natural, therefore, to look expectantly into the contents of the latrine in hope of finding direct evidence of the use of herbs. There are indeed some seeds of such plants as self heal (Prunella vulgaris) and black nightshade (Solanum nigrum), quite apart from the fig, of course. But some of the finds may give cause for concern, such as the deadly nightshade (Atropa belladona) and water dropwort (Oenanthe croccata) and hepbane (Hyoscyamus niger). In view of the toxicity of these species, one cannot help wondering what eventually befell the user of the latrine.

A sad, though predictable, feature of the analysis is the high concentration of parasite eggs *Trichuris* (whip worm) and *Ascaris* (roundworm), with the former predominating in the ratio 3:1. This reflects the generally poor living conditions and lack of hygiene at that time.

From the contents of this barrel, Greig has managed to piece together an intriguing and complex picture of everyday medieval life in Worcester and may have purged some of the disinclination to exploit the archaeological potential of such residues.

# ARTICLES

# Volcanic earthquakes at Pavlof Volcano correlated with the solid earth tide

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Volcanic earthquake swarms at Pavlof Volcano, Alaska, correlate significantly with solid earth tidal stress rate for periods just before and just after explosive eruptions. The correlation changes sign systematically during the course of the 1974 minor eruption sequence; pre-eruptive earthquake swarms occur during increasing tidal compression, while post-eruptive swarms occur during increasing tidal extension.

IT has been proposed that the solid earth tide has a role in triggering both earthquakes and volcanic eruptions (see refs 1-5). Crustal stresses of the solid earth tide are of the order of 0.03 bar, with principal periods of ~12 h 25 min, 24 h 50 min, and 14.7 days, and constitute some of the largest pervasive short period oscillatory stresses in the Earth's crust<sup>6</sup>. We examine here the relationship between volcanic microearthquakes and the solid earth tide during the autumn 1974 minor eruption of Pavlof Volcano, Alaska. A correlation has been found between B-type<sup>7</sup> volcanic earthquake swarms and solid earth tidal stress for periods of 3-4 days before and after eruptions. The correlation changes sign systematically for time periods before and after the eruptions, corresponding to periods of presumed inflation and deflation of the volcano.

Pavlof is a 2,715-m high stratovolcano located near the end of the Alaska Peninsula at 55°25′ N, 161°54′ W. It is one of the most active volcanoes in North America with at least 27 documented eruptions since 1700 (S. Hickman, personal communication). Strombolian eruptive activity typically includes lava fountaining and flows, emission of steam and ash, and minor explosions. Analysed lavas are basalt, with small phenocrysts of plagioclase, olivine, hypersthene and augite<sup>8</sup>. The upper portion of the mountain is covered by glaciers.

Twelve seismometers are in operation near Pavlof (Fig. 1). BLH and PVV are stations of the Shumagin Islands regional network, operated since 1973 by Lamont-Doherty in cooperation with the University of Alaska. The remaining local network stations were installed by Lamont-Doherty in 1976, that is, after the sequence of eruptions discussed here. Most of the data presented here are from station PVV, a short period (1 Hz) vertical seismometer located adjacent to an old lava flow 7.5 km south-east of the volcano's summit. The data are recorded on paper helicorder records.

The autumn 1974 eruption sequence began with minor explosive activity from 2 to 21 September, followed by a period of repose. Explosive activity occurred again from 29 October to 19 November, 25 November to 16 December and 25 December to 6 January. Each of these three periods of explosive volcanism began 3-4 days after fortnightly neap tides, in close agreement with Johnston and Mauk's previous results from Mt Stromboli, Italy.

	Table 1	Pavlof volcano seismicity				
Period		Mean no. of events per 2 h	s.d.	No. of data points		
Non-eruptive		10.05	5.65	96		
Pre-eruptive		26.74	18.81	114		
Eruptive		20.24	13.58	178		
Post-eruptive		14.19	13.53	161		

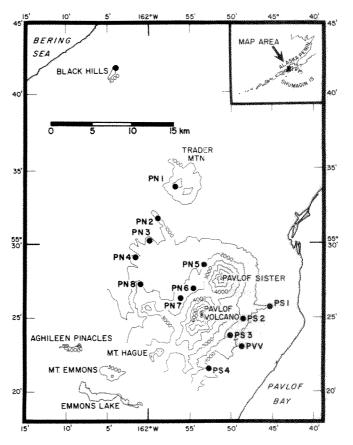


Fig. 1 Map showing stations of the Pavlof seismic array. Data are from station PVV, 7.5 km to the south-east of the volcano's

#### Seismicity

Data reduction consisted of detailed counts of each of four different types of volcanic earthquakes: high frequency tremors, harmonic tremor, explosion earthquakes and B-type earthquakes ranging in magnitude from -0.2 to 1.0 (Fig. 2) (S. McNutt in preparation). The numbers of events of each type per 2-h interval were plotted on histograms (see Fig. 3) for the entire time period from 1 September 1974 to 30 April 1975. Table 1 gives statistics for the general seismicity, broken down into four periods: non-eruptive (background), pre-eruptive, eruptive and post-eruptive. Seismicity is highest during pre-eruptive periods, followed by eruptive, post-eruptive, and background periods.

Table 2 Number of B-type events versus tidal stress rates in certain azimuths

A	Azimuth	0°	45°	90°	135°	Volumetric	N
Non-eruptive	14–16 January	0.17 $-0.21$	0.19 + 0.04	$0.19 \\ -0.46$	$0.14 \\ -0.22$	$0.20 \\ -0.26$	36
Eruptive	4-8 November	0.02 -0.04	0.11 +0.01	0.09 -0.26	0.00 0.00	0.05 $-0.11$	42
Pre-eruptive	29 October-1 November	0.45 -1.24	0.49 -1.67	0.49 -2.48	0.41 -1.45	0.51* -1.50	37
Post-eruptive	18-21 November	99% 0.22 +0.50	99% 0.22 +0.56	99% 0.33 +0.86	99% 0.31 +0.72	99,9% 0.29 +0.56	46
Pre-eruptive	22-26 November	0.25 -0.82	0.32 -1.25	98% 0.38 -2.16	95% 0.26 -1.05	95% 0.33 -1.10	43
Post-eruptive	7–12 January	0.35 +0.93 99%	95% 0.36 +1.09 99%	98% 0.34 +1.26 99%	0.32 +0.98 98%	95% 0.37* +0.93 99%	60

The upper value of each entry is the correlation coefficient, the second is the normalized slope and the third is the confidence level estimate for those cases where it is greater than 90%. N is the number of data pairs used.

\* The data plotted in Fig. 4.

Eruptive periods are so designated on the basis of the presence of explosive activity.

B-type earthquakes (Fig. 2) at Pavlof are nearly identical to those observed elsewhere  $^{7,10}$ . They have emergent onsets, no clear S-phases, dominant frequencies of  $\sim 1.4$  Hz, and coda durations of  $\sim 20$  s. Other studies in Japan have located B-type earthquakes at extremely shallow depths (1 km) immediately adjacent to active vents. The Gutenberg-Richter magnitude frequency relation for 1,316 B-type earthquakes which took place from 1 to 8 December has a negative slope or b-value of  $2.6 \pm 0.2$ , in close accord with values calculated for other volcanoes 1.0.1 Explosion earthquakes (Fig. 2) closely resemble B-type earthquakes, except for an impulsive spike  $\sim 21$  s after the signal onset. This spike is the air shock phase.

#### Tidal computations

The earthquake histograms were compared with a synthetic solid earth horizontal stress tide in azimuths 0°-180° and with volumetric stress. The components of the tide as a function of time and position were calculated by Longman's method 12, using a program which agrees closely with independent computations. Longman's method calculates tidal potential, from which stress is easily derived by differentiating and using the stress-strain relations with standard values of Love numbers and Lamé parameters. Visual inspection showed that earthquakes tended to occur at times of increasing or decreasing stress in certain azimuths. We therefore differentiated the synthetic stress tides with respect to time to generate stress rate tides (Fig. 3).

The ocean loading tide must be accounted for as well as the solid earth tide; for the particular case of Pavlof, the ocean load stress tide is approximately in phase with the solid earth tide. A rough calculation using Farrell's  $^{13}$  Green's functions and Schwiderski's  $^{14}$  ocean tide charts shows that for volumetric stress and for stress in azimuth N90° E the load tide maxima are within 1 h of the solid earth tide maxima. The load tide amplitude is  $\sim 30\%$  that of the solid earth tide; hence the inclusion of the load tide changes the phase by <1 h. We do not consider here the detailed effect of topography on the stress tide, but note that topography normally has a small effect on the phase  $^{15}$ .

#### Correlation data

Linear correlation coefficients between number of earthquakes per 2 h period and tidal stress rate for 15° increments in azimuth were calculated for portions of the eruption sequence. Figure 3 shows the number of earthquakes and two components of stress rate plotted against time for two pre-eruptive and two posteruptive portions of the data. The third post and pre-eruptive

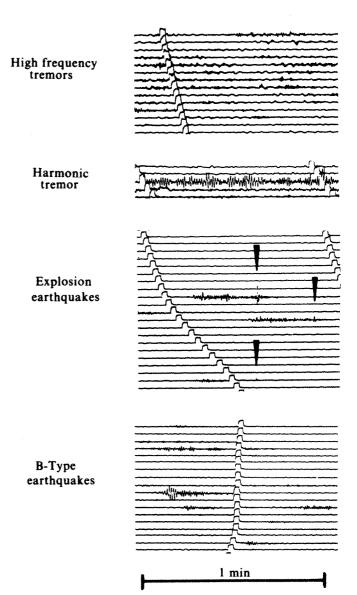


Fig. 2 Types of seismic signals recorded on helicorder from station PVV. Arrows point to air shock phase of explosion earth-quakes. Note the similar frequency content ( $\approx$ 1.4 Hz) of B-type earthquakes, harmonic tremor, and ground waves from the explosion earthquakes.

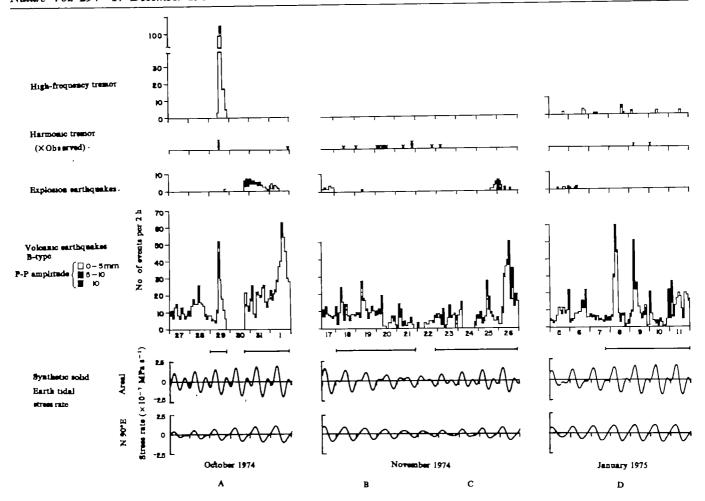


Fig. 3 Portions of histograms showing numbers of volcanic earthquakes of each type compared with synthetic solid earth tidal stress rate. A positive stress rate indicates increasing extensional stress. Note in particular the relation between the number of B-type earthquakes and tidal stress rate. A and C label pre-eruptive periods, while B and D label post-eruptive periods. During periods A and C peaks in the number of B-type earthquakes tend to occur at negative peaks in stress rate, while during periods B and D earthquakes occur at positive peaks in stress rate. See text for fuller explanation. The horizontal bars between the histograms and the tidal data show the portions of data actually used in the correlation calculations.

sequence is not shown; seismicity during this time period was the highest for the whole 7-month study period, and for reasons given below the results are less reliable. The results given in Table 2 show no statisticially significant correlation with tidal stress rate for either non-eruptive or eruptive seismicity. However, pre-eruptive and post-eruptive seismicity do show statistically significant correlations between the number of Btype volcanic earthquakes and the solid earth tidal stress rate for certain azimuths (Fig. 4). During pre-eruptive and post-eruptive periods earthquakes tend to occur at the times of tidal zerocrossings, that is when the rate of change of the tidal stress is most rapid. During pre-eruptive periods the correlation is negative: B-type earthquakes tend to occur at the time of most rapid increase in compressional tidal stress. For post-eruptive periods the correlation is positive: earthquakes tend to occur at the time of most rapid increase in extensional tidal stress. Table 2 shows a small but representative sample of the computed correlation data: estimates of correlation coefficients of regression, normalized slopes (that is, numbers of earthquakes per unit stress rate) and the confidence level of the correlation (the probability that the true correlation is greater than zero) are listed. We find that correlations with horizontal stress rate are better for azimuths from 75° to 105° than for other azimuths; correlations with volumetric (or areal) stress are similar to the best horizontal stress correlations.

#### Discussion

Ryall et al. 16 stated three criteria that should be met to evaluate optimally a tidal correlation: a statistically significant number of

events, a limited focal region, and a relatively constant character of accumulated strain in the region, as evidenced by focal mechanism study of the earthquakes. The first two conditions are met at Pavlof Volcano. Focal mechanisms could not be determined for the volcanic earthquakes because (1) it is nearly impossible for volcanic earthquakes to determine polarity of the emergent first arrivals, and (2) only one station was in existence at the time of this sequence of events; however, the signal characteristics are remarkably similar and are consistent with a relatively constant mechanism. Several other factors are important: the eruption was small, hence processes were smooth and slow rather than catastrophic; except for one time period (16-25 December) the seismicity was low enough that there was virtually no overlap problem in reading records; and the earthquakes themselves were small, thus the tidal stresses may have attained a larger fraction of the presumed stress drops (~a few bars) of the earthquakes than is the case for catastrophic events.

Our data are insufficient to determine unambiguously the focal process responsible for causing the earthquakes. Two processes are most likely; mode I (tensional) cracks and mode II (shear) cracks, which are both thought to occur as a result of magma injection into the volcanic pile. For the same crack geometry, however, the two processes would require different orientations of the principal stress axes to enhance most crack growth, or, vice versa, for a given stress system the two types of cracks most likely to be activated would be oriented at different azimuths. Our correlation data suggest a preferred orientation of cracks, but we cannot determine this orientation uniquely. Determinations of maximum horizontal compressive tectonic

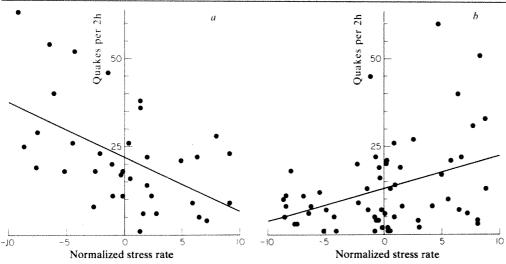


Fig. 4 Linear regression fits between earthquake frequency and stress rate. a, The data for a pre-eruptive period where the correlation is negative. The correlation coefficient is 0.51, and is >0 at the 99.9% confidence level. b, A post-eruptive period where the correlation is positive. The correlation coefficient is 0.37, and is >0 at the 99% confidence level.

stress<sup>17,18</sup> and the azimuth of relative plate convergence of the North American and Pacific plates 19 suggest an azimuth for the P-axis of maximum compression of approximately 150° which may control preferred crack growth in this direction if tensile cracks dominate, or at some angles ±45° from this azimuth if shear cracks dominate. This discussion does not consider pore pressure variations, which could change the effective stress significantly.

We tentatively interpret the change in sign of the correlation in terms of inflation and deflation of the volcano before and after eruptions, respectively. Some volcanoes tend to inflate before and deflate after eruptions<sup>7,20</sup>, presumably due to buoyant forces exerted by magma on the volcanic edifice as the magma migrates through dykes, but no geodetic data are available for Pavlof to confirm this hypothesis.

The tidal correlation applies only for a period of 3-4 days before and after explosive eruptions. As in previous studies<sup>2</sup> this implies that the volcano is sensitive to changes in ambient stress rate of  $\sim 2.5 \times 10^{-7}$  MPa s<sup>-1</sup> during the times when magma

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is near the Earth's surface. Other volcanoes have quite slow rates of tectonic stress accumulation before eruptions<sup>20</sup>. The time period of 3-4 days may represent the time of magma ascent from or descent to a shallow magma chamber.

Our results indicate that the earth tide may have an effect on certain aspects of volcanic activity at Pavlof. This paper is believed to be the first to show a tidal correlation whose polarity with respect to tidal phase varies systematically during a volcanic eruption.

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# Lithospheric studies based on holographic principles

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Earth holography was applied to Norwegian Seismic Array (NORSAR) earthquake P-wave recordings. Simulation of the NORSAR sensor configuration indicates that a satisfactory reconstruction of the essential features of an embedded body is feasible with a depth resolution which seems to be better than that attained using more traditional time and amplitude inversion schemes. The earth holography concept was tested using NORSAR recordings of three deep earthquakes at teleseismic distances. The results indicate lithospheric heterogeneous bodies with a significant vertical extent. The most pronounced scattering features of these inhomogeneities are located at depths around 100, 148 and 212 km.

DETAILED mapping of subsurface geological structures is important both for exploration for natural resources and for a better understanding of tectonic processes. The best tools are those tied to seismic wave analysis in view of the very short wavelengths involved compared with other geophysical investigations. During the 1970s two novel approaches were introduced: (1) the scattering approach using the concept of an isotropic random medium with a prescribed statistical velocity perturbation, correlation distance and turbidity factor<sup>1-3</sup>; and (2) the ACH method<sup>4-8</sup> which inverts travel time observations of a deterministic model into velocity perturbations. These approaches proved successful both in terms of three-dimensional seismic images of subsurface structures and in explaining precursor wavetrains<sup>9</sup> in the seismogram. A viable alternative might be to introduce a deterministic scattering model, assuming the presence of discrete inhomogeneities.

The surface wavefield manifestations of such inhomogeneities are in terms of diffraction effects which leads to an inverse diffraction problem for their mapping. This is analogous to the image reconstruction problems of holography<sup>10,11</sup>.

In applying holographic principles to analysis of seismological observations (earth holography), direct travelling seismic waves should be used because inhomogeneities often cause strong forward scattering. For example, Fig. 1 shows what the image of different structures should be when 'seen' by passing seismic waves. Compared with optic and acoustic holography, earth holography poses practical problems: complicated structure, the scale of the heterogeneities relative to that of useful seismic wavelengths is small, aperture of the sensor array is only of the order of 10–20 times the seismic wavelength as compared with a factor of  $10^7$  in optics, relatively poor wavefield sampling and background noise.

We now describe an attempt towards applying the principles of holography on a seismological scale. To test thoroughly the impact of the limiting factors an ultrasonic modelling experiment was undertaken to simulate the sampling of the NORSAR array in Norway<sup>12</sup> comprising 132 short period vertical seismometers (up to 1976).

#### Theoretical background

Despite extensive applications of the principle of wavefield reconstruction, its mathematical foundation has been formulated only recently 11,13-15 and the wavefield reconstruction has been reduced to a boundary value problem associated with the scalar wave equation.

The solutions of the forward and inverse diffraction may be written in similar forms using appropriate Green's functions:

$$U(x_2, y_2, z_2) = \frac{1}{2\pi} \int_{-\infty}^{\infty} U(x_1, y_1, z_1) \frac{\partial}{\partial z_1}$$

$$\times \left(\frac{\exp[-jkr]}{r}\right) dx_1 dy_1 \tag{1}$$

$$U(x_1, y_1, z_1) = -\frac{1}{2\pi} \int_{-\infty}^{\infty} U(x_2, y_2, z_2) \frac{\partial}{\partial z_2}$$

$$\times \left(\frac{\exp[jkr]}{r}\right) dx_2 dy_z \tag{2}$$

where for the forward problem the distribution of U (pressure or velocity fields) at plane  $z_2$  results from the knowledge of the distribution in the  $z_1$  plane  $U = U_1(x_1, y_1, z_1)$ , with  $z_2 > z_1 > 0$ . In the inverse problem the distribution of the wavefield in plane  $z_1$  is determined from knowledge of the field distribution on plane  $z_2$  (equation (2)). k is the wavenumber of the U-field and r is the distance between points in planes  $z_1$  and  $z_2$ .

A possible computational procedure for solving equation (2) follows from the convolutional nature of this equation, and is based on the FFT algorithm. Another technique which is adopted here is to take the derivative under the integral of equation (2) and assuming  $k \gg 1/r$  we then get:

$$U(x_1, y_1, z_1) = -\frac{j}{\lambda} \int_{-\infty}^{\infty} U(x_2, y_2, z_2) \frac{\exp[jkr]}{r}$$

$$\times \cos \theta \, dx_2 \, dy_2$$
(3)

where  $\theta$  is the angle of incidence. The approximation of equation (3) in combination with equations (1) and (2) is reasonable for the present models.

Further simplifications are feasible by a series expansion of r in the exponent of equation (3) and by retaining only the first two terms. This would be analogous to the Fresnel approximation used for solving forward diffraction problems.

#### Ultrasonic modelling experiment

The set-up of the ultrasonic modelling experiment is outlined in Fig. 2a, where the 'transparent' or homogeneous body is a parallelepiped made from epoxy with an embedded aluminium cross serving as the inhomogeneity whose image is sought reconstructed by holography. A monochromatic wave frequency of 880 kHz was used which is equivalent to  $\sim 1.8 \text{ Hz}$  in the seismological case. The choice of the frequency 1.8 Hz was motivated from the modelling experiment itself, implying an upper limit of  $1.3 \lambda$  for grid spacing. This is equivalent to  $\sim 4 \text{ km}$  in the seismological case (using a mantle velocity of  $8.2 \text{ km s}^{-1}$ ) which in turn reflects the NORSAR subarray sensor interspacing of 2-4 km. The major modelling parameters are also

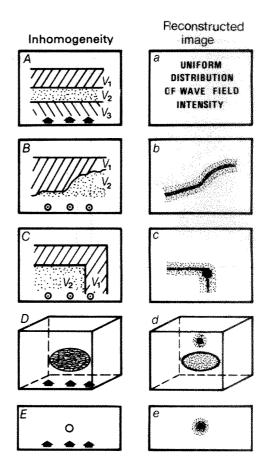


Fig. 1 Realistic earth inhomogeneities and their anticipated reconstructed holographic images. For A, D, E the illuminating plane-wave source is beneath the inhomogeneity while for B, C the source is located perpendicular to the plane of the figure. In A/a the reconstructed image would have uniform intensity so the inhomogeneity would appear transparent, thus contrasting sharply with conventional profiling results. For D/d the ghost image at intermediate depth reflects the smaller wave velocity of the object. The small single scatter object in E/e would probably be lost in a profiling survey.

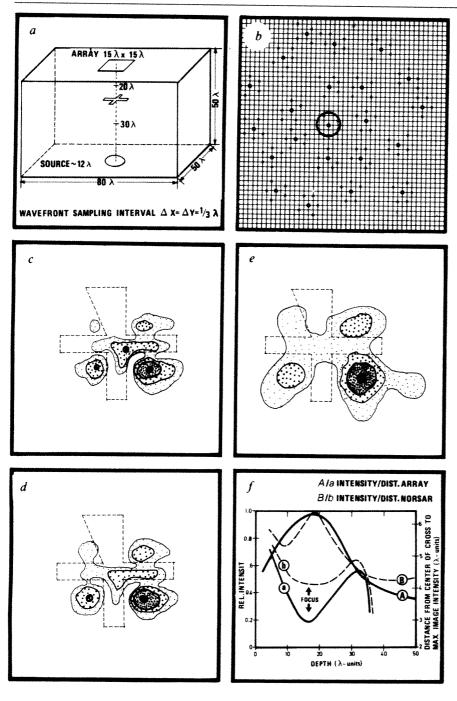


Fig. 2 Ultrasonic experimental set-up and results. a, The 'transparent' medium in the form of an epoxy box  $(v_p = 2.6 \text{ km s}^{-1})$  and the embedded inhomogeneity in the form of an aluminium cross ( $v_p = 5.2 \text{ km s}^{-1}$ ). The size of the box is  $80 \times 50 \times 50 \lambda^3$  while the standard length and width of the individual arms in the cross are  $\sim 12\lambda$  and  $\sim 2\lambda$  respectively. b, The two sensor configurations used: (i) a rectangular grid with 2,025 sensors with an interspacing of  $\lambda/3$  and (ii) a 132-sensor geometry similar to that of the NORSAR array. c, Image reconstruction of the cross (outlined) at its correct depth location on the basis of the regular grid network with contributions from all, 2,025 sensors to each reconstructed point. Relative intensity scaling is used with contour levels at 1, 2, 4 and 8 dB. d as c except that contributions are restricted to those observational points being within a radius equal to the diameter of the first Fresnel zone. The relatively high intensities observed at the centre of the cross in c and d stem from the non-plane source illumination. e, Image reconstruction of the cross when the observational points were limited to a simulated NORSAR array configuration of 132 sensors which in turn were extrapolated over a grid of  $25 \times 25$  points with 0.6  $\lambda$  interspacing. As in d contributions to each reconstructed point were limited to a radius equal to the diameter of the first Fresnel zone. f, Relative intensity as a function of image reconstruction depth for both configurations of b. The strongest intensity is found at the proper depth location of the cross and this coincides with a minimum distance from centre of the reconstruction axis. The intensity estimations for depth exceeding  $\sim 30\lambda$  reflects reconstruction of the source itself. The intensity maximum at the depth of the cross and the coinciding centre axis minimum distance both imply that depth resolution should be substantially better than that of three-dimensional time inversion<sup>4.5</sup>.

indicated in Fig. 2a; the epoxy block dimensions would correspond to an upper mantle block of roughly  $300 \times 300 \times 500 \text{ km}^3$ . Wavefront amplitude a(x, y) and phase  $\phi(x, y)$  were recorded across the sensor aperture area in 2,025 sampling points. By recording phase information, the reference wavefield required in optics is superfluous. Also, using computerized image reconstructions the three-dimensional 'view' typical of optic holography is substituted by two-dimensional cross-sections at different levels. This experiment is also considered adequate for simulating the NORSAR sensor configuration (Fig. 2b).

The cross-image reconstruction results are displayed in Fig. 2c-e for the two sensor configurations shown in Fig. 2b, namely, the whole grid of 2,025 sensors (Fig. 2c, d) and that of simulating the 132 sensor positions of the NORSAR array (Fig. 2e). The cross-images in the upper left quadrants of Fig. 2c-e are not entirely satisfactory and this prompted a reexamination of the experimental design. It was discovered that the source-positioning was slightly out of focus (lower right quadrant) and the source radiation was not plane but of a complicated spherical form. Taking these deficiencies into account, enables the pecu-

liarities in Fig. 2c-e to be explained rationally. For example, the strongest intensity is found in the lower right quadrant reflecting the asymmetric positioning of the source itself. The non-plane source radiation effect gave rise to focusing effects in the range  $16-23 \lambda$  below the array surface. This explains why the outer cross corners are not seen, and the existence of the centrally located intensity peak in Fig. 2c.

The results in Fig. 2c-e indicate that even in the case of gross holographic undersampling of the wave field, in particular for the simulated NORSAR configuration of 132 sensors only, a satisfactory reconstruction of the essential features of an embedded body seems feasible.

Another important aspect of the earth holography is that compared with other methods its depth resolution is probably very good. From optics the depth resolution would be  $2\lambda$  and  $1\lambda$  vertically and laterally for ideal cases. In the ultrasonic experiment the vertical depth resolution appears to be within  $3\lambda$  (Fig. 3f) which is highly satisfactory. In practical earth holography the depth resolution is likely to be less impressive, say, within  $5\lambda$  due to the relative complexities of lithospheric materials, but would still compare favourably with that obtained through

travel-amplitude inversion experiments. Other factors affecting the resolution in a rather complex way are array aperture, sensor spacing and depths to the anomalous bodies<sup>16</sup>.

#### Lithospheric heterogeneities

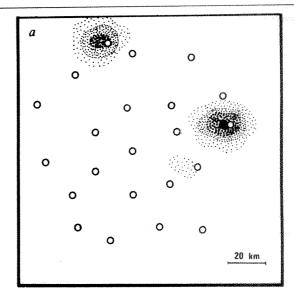
For testing the earth holography concept, NORSAR P-wave recordings of three deep earthquakes at teleseismic distances (South of Honshu, Hindu Kush and Western Brazil) were analysed. A characteristic feature of these recordings is that each of the 132 seismograms for one event exhibit an interference pattern of pulse-shaped waves propagating through the receiver region at slightly different paths. The first arrival is the direct (refracted) wave while scattering contributions arrive a little later. Obviously, the main information on lithospheric heterogeneities is confined to the initial part of the seismogram—here set to the first 10 s. The information extraction was tied to (spectral) amplitudes and phases for several harmonic components (0.6-2.6 Hz) of the recorded signals. However, as the 1.8-Hz component proved to be best, the results are restricted to this frequency. Note that the monochromatic signals of optical holography can be simulated by using a wideband frequency (or pulse) source in combination with sensors having resonant characteristics. The latter design is obtainable using digital filtering in time or frequency domain. Also, for image reconstruction the Earth's crust was given a thickness of 36 km and an average P-velocity of 6.5 km s while the lower part of the lithosphere was a half space with a P-velocity of 8.2 km s<sup>-1</sup>. Because this analysis is extremely computer-time consuming and each additional event only provides an independent set of reconstructed images the analysis was restricted to three clear event recordings.

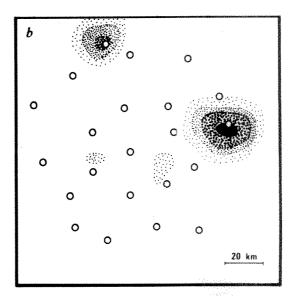
Results tied to image reconstruction of lithospheric heterogeneities under NORSAR using the south of Honshu earth-quake recordings are given in Fig. 3, which shows only the most striking features. At a depth of 100 km (Fig. 3d) the prominent intensity maximum to the east was the most concentrated one found over 14 intervals in the depth range 0-250 km. This bright spot also has the closest location relative to the centre of the array (see Fig. 2 legend).

For the anomaly to the north a similar effect is attained at a depth of 148 km (Fig. 3b). As depth increases the two anomalies deteriorate while two weak anomalies near the centre of the array emerge (Fig. 3c). Composite results for all three events analysed are shown in Fig. 4 for the same depth intervals as used previously. Area I is a rather weak anomaly at a depth of 148 km which is not clearly 'seen' by the western Brazil event; area II is the brightest heterogeneity of shallow depth (~100 km) which gives energy maxima for all three events; area III-a rather strong inhomogeneity—is located near the centre of the array, and is most clearly seen in the western Brazil results. The three events indicate different depth locations for areas I, II and III (III being the deepest), and that suggests a significant vertical extent of the heterogeneous bodies. These are 'illuminated' differently by the respective events, for example, side scattering by a rather strong heterogeneity seems to be required for anomaly II to be seen by the Western Brazil event. For areas IV. V and VI the anomalies are rather weak and are seen most clearly by the Western Brazil event.

#### Discussion

Much research effort has been devoted to analysing the complex P-wave field observed across NORSAR, and both random medium and derministic<sup>4,5</sup> approaches have been utilized. The latter result, the essence of which is indicated in Fig. 4 in terms of relative P-velocity anomaly contours, is the most comprehensive obtained so far and is a natural choice for a comparison with the outcome of the holography experiment, although a one-to-one overlap between the inversion results cannot be expected; time inversion has relatively poor depth resolution whereas earth holography results depend partly on the direction of the wave approach. Nevertheless, the relatively marked image intensities of areas II and III coincide reasonably well with the strong





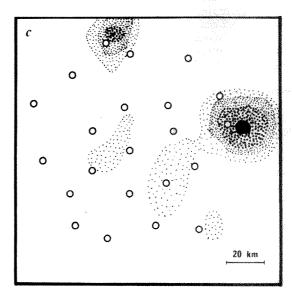
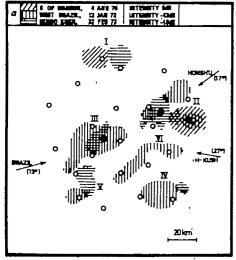
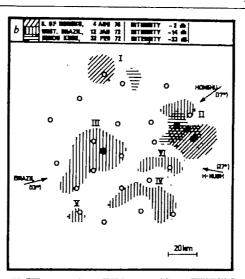
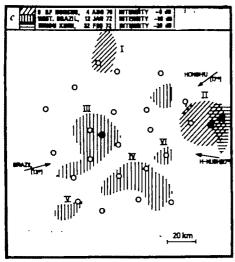


Fig. 3 Image reconstructions at depths of: a, 100 km, maximum intensity = 0 dB; b, 148 km, maximum intensity = -1.8 dB; and c, 212 km maximum intensity = -7.5 dB for the south of Honshu event using the computational procedure as indicated in Fig. 2e legend, frequency = 1.8 Hz. The intensity grading in each figure is in steps of 1 (black), 2, 4 and 8 dB down from maximum. The strongest intensity is to the east in a and is also the most concentrated. Note that this intensity has the closest location vis-a-vis the array centre and in this respect is similar to the focusing effect in Fig. 2f.







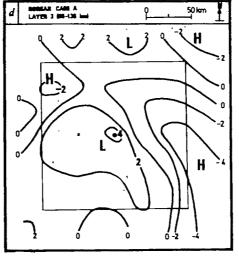


Fig. 4 Composite display of intensity maxima for depths. a, 100 km; b, 148 km; and c, 212 km exhibiting the most pronounced scattering features. frequency = 1.8 Hz. Different shading is used to identify the three events and their relative intensity scaling is also given. The contours of the various shaded areas are 4 dB down from the individual event maxima. A good overlap in a between the intensity areas for the different events implies that heterogeneities at this depth are most uniformly seen by the three events. b, Which has weaker intensities than a, also exhibits less of an overlap between the respective event intensity areas. Similar comments apply to a. The consistency in location of the intensity areas L II and III between a, b and c implies a significant depth extent of the heterogeneous bodies. For comparison the P-residual inversion results' in terms of relative velocity perturbations in per cent for model A of ref. 5, depth interval 85-135 km are displayed in d. There is reasonable agreement between these two types of seismic results. Note that the time inversion results for the depth interval 185-235 km are essentially the same as those for the 85-135 km interval, implying decreasing resolution with increasing depth with this method.

velocity anomalies obtained by direct inversion of travel time residuals. The intermediate image intensity of area I does not have a clear counterpart in the residual inversion results, and the same applies to the weaker area VI. In both these cases pronounced velocity changes occur nearby. The weak area V, may actually belong to area III as both are within the pronounced velocity low in the southwestern quadrant. The area IV inhomogeneity which is also weak may be associated like area II with the velocity high to the east. Area VI which is seen only by the Western Brazil event may be very deeply located (say > 200 km) and by that poorly resolved in view of the array's aperture of only 100 km.

We now consider the results of Fig. 4 in terms of geological structures. The fact that none of the anomalous areas observed seem to originate at shallow depth (≤80 km) is not unexpected as, with NORSAR's rather coarse sampling, the crust would appear relatively transparent. Thus, no obvious correlation was found with surface geological features such as the Oslo graben nor with a mylonite to the south-east. Except for the seismic manifestation of the Oslo graben<sup>5</sup> weak impedance contrast this agrees well with time residual inversion results. The anomalous body corresponding to the imagery reconstructions of areas I, II and III seems to represent bodies of different impedance contrasts and also different vertical extent. However, from the implied vertical extent of the heterogeneities corresponding to areas I. II and III we conclude that predominantly vertical processes take place within the lithosphere 17 and in particular in association with taphrogenesis18.

The interpretation of the results displayed in Figs 3 and 4 depends on the depth resolution of the associated anomalous areas which was discussed above with basis in optics. In earth holography the conditions are more difficult with noise in the

observations, the probable vertical extent of the anomalies and the general complexities of lithospheric structure. However, our previous estimate of  $5\lambda$  in the vertical direction seems to be confirmed by the intensity depth sharpness of the anomalous areas I, II and III and in this respect is substantially better than that obtained in the inversion experiments. For example, the Fig. 4f time inversion anomalies for the depth interval 85-135 km remain essentially unchanged when projected 100 km lower down<sup>5</sup>. The same applies to amplitude inversion experiments7.

#### Conclusions

We have demonstrated that acoustical holography can be applied to seismological observations from a large aperture array such as NORSAR. The results obtained in terms of located heterogeneous bodies in the lithosphere are in reasonable agreement with inversion results based on time residual observations from 183 events even though only three events were used for this analysis. Also, the depth resolution of our earth holography approach seems to be much better than that of the inversion technique and is estimated to be within  $5\lambda$  or 20 km in this particular case. The earth holography technique uses deterministic scattering effects often ignored in traditional seismic investigations and thus provides important, supplementary information on lithospheric heterogeneities.

The earth holography technique could be used on scales comparable with those used in seismic prospecting say for locating ore bodies, fault mapping and other sources of strong diffracted waves. However, the necessity of sufficiently dense sampling restricts its practical usage at present but its future application should be possible with improvements in digital

recording techniques.

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# Base pairing of RNA I with its complementary sequence in the primer precursor inhibits ColE1 replication

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Four single base pair mutations in the region that codes for RNA I create new incompatibility groups while preserving the mechanism of control of initiation of DNA replication in a ColE1-type plasmid. Sequence analysis of these base substitutions points to the primary importance of the central loop of the cloverleaf structure of RNA I in this control mechanism.

PLASMIDS offer an attractive model for investigating the molecular mechanisms that ensure controlled replication of bacterial replicons during each division cycle. In many plasmids two species of RNA molecules, coded for in the region of the replication origin, seem to have a crucial role in the control of plasmid replication<sup>1-6</sup>. One of these, RNA II, is transcribed from a point upstream from, and towards, the replication origin, terminating at a point beyond it. In ColE1-like plasmids RNA II molecules have been shown in vitro to be processed at the origin of replication by RNase H (ref. 3) to form primers for the initiation of DNA synthesis by DNA polymerase I. The second, and smaller, RNA species, RNA I, is transcribed in the opposite direction to RNA II from the region of the plasmid genome shown genetically to determine plasmid copy number<sup>1,2,5</sup>. In vitro, RNA I molecules prevent the maturation of RNA II molecules, in a way that is not understood<sup>6</sup>. RNA I only inhibits primer formation by the plasmid that codes for it, having no effect on heterologous plasmids<sup>6</sup>, and it has been proposed that this interaction controls plasmid copy number. Here we show that single base pair substitutions in the region complementary to the central loop of RNA I's putative cloverleaf structure create new compatibility groups (defined by replication of the mutant in bacteria harbouring plasmids with homologous replication origins) by changing the specificity of the target of RNA I and suggest a model for the inhibitory interaction.

#### Isolation of target mutants

We expected that mutants altered in the target of the inhibitor of plasmid replication would be dominant high copy number mutants. However, rather than using direct selection for plasmids with a higher number of copies, we used an approach involving the phasmid system<sup>7</sup>, so as to avoid overlooking mutant plasmids potentially lethal for the host and to select against recessive mutants. This approach is particularly suitable for genetic analysis and relies on the possibility of incapsidating plasmid sequences in a  $\lambda$  phage head (Fig. 1).

We have previously observed<sup>7</sup> that the insertion of the plasmid pacl29 (Fig. 3) into the  $\lambda$  chromosome by int-mediated recombination confers on the hybrid the ability to grow on a homoimmune lysogen. This virulence is due to titration of  $\lambda$ 

repressor by the extra copies of  $\lambda$  operators that result from replication of the hybrid using the plasmid origin. The presence in the lysogenic strain of a resident plasmid that synthesizes the inhibitor of plasmid replication prevents virulence by blocking the initiation of plasmid DNA synthesis in the incoming phasmid. A phasmid with an altered inhibitor target will be able to escape repression and will be virulent even in the presence of a resident plasmid (Fig. 1a).

The isolation of such inhibition-insensitive mutants (svir) will be described in detail elsewhere<sup>8</sup>; here we summarize their properties. Six independent svir mutants were isolated. Only four of them can be released efficiently from the  $\lambda$  chromosome by infection of an int constitutive strain (Fig. 1b); the remaining two mutants, svir3 and svir19, are lethal for the host and cannot be propagated as plasmids. Three of the mutant plasmids that replicate efficiently show an increase in relative copy number with respect to wild type (Table 1). This increase is dominant in a complementation test. Unexpectedly, the remaining mutant, svir11, is present in the host bacterium with a lower number of copies than the wild type (0.7 relative copy number), as if the mutation had created a better mechanism of self repression. From the behaviour of these mutants in compatibility experiments we conclude that the target of the inhibitor of ColE1-type replication coincides with part of the inhibitor coding sequence. As a consequence, single mutations in the target result in altered inhibitors that are unable to interact with the wild-type target. This hypothesis is consistent with what is known about the transcriptional organization of the replication region of ColE1 and the finding that RNA I inhibits primer formation by acting somewhere further upstream than 300 nucleotides from the

#### Specificity test

If svir plasmids are mutated not only in the target but also in the repressor, we are left with the puzzle of the low copy number of svir11. One possible explanation would be that a single base pair change had modified target and repressor in a complementary way that maintained and possibly improved the interaction between the two mutated elements. To test this hypothesis we devised the test shown in Fig. 2 where each svir phasmid

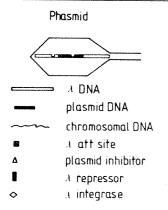
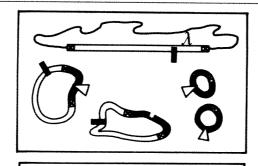
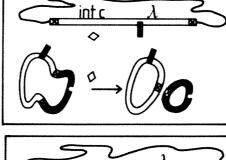


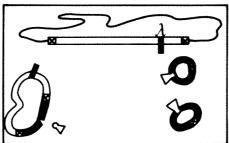
Fig. 1 The phasmid system. The drawings illustrate schematically how a phasmid can be used to select dominant mutants in the target of the negative regulator of plasmid replication. The phasmid<sup>7</sup> is a hybrid obtained by in vivo integration into the  $\lambda$  chromosome of a plasmid containing an att site. The resulting hybrid molecule can be encapsulated into  $\lambda$  heads and, as a consequence, plasmid sequences can be injected into different bacterial environments by phage infection. Infection of a lysogen containing a plasmid of the same compatibility group (a) will not lead to any phage production. Both phage and plasmid replication system are, in fact, blocked. Phasmid mutants carrying an altered target for the inhibitor of plasmid replication can replicate under plasmid control and start a lytic cycle when the copy number reaches a value sufficient to titrate-repressor. The process of plasmid integration is reversible and plasmid sequences can be excised from the  $\lambda$ chromosome (b) by infecting the strain EQ84 =



a Supervirulence test.
Only phasmid with altered target for plasmid inhibitor will be able to replicate efficiently



b Release of plasmid Excision of plasmid sequences in integrase constitutive strain



c Specificity test.Do mutant plasmid repressors crossreact with mutant targets?

HfrH galT  $\Delta$ (int-FII) ( $\lambda$  inte 226) that constitutively synthesizes  $\lambda$  integrase. Clones that contain the original plasmid sequences can be selected at 30 °C on plates containing 50  $\mu$ g mI<sup>-1</sup> of ampicillin. The experimental details of the specificity test (c) are described in Fig. 2 legend.

(horizontal lines) was spotted on a lawn containing a permissive indicator together with a lysogenic strain harbouring the different *svir* plasmids (vertical lines). A turbid spot indicates that the phasmid is unable to replicate in the corresponding lysogenic strain. Insensitivity to the inhibitor synthesized by the resident plasmid will result in phasmid replication, virulence and, as a consequence, in a clear spot. Experiments of the type shown in Fig. 2 clearly indicate that not only *svir11*, but also the three high copy number *svir* mutants, can still synthesize an active inhibitor. Furthermore, the turbid spots are present only in the diagonal, indicating that the mutant inhibitors are active only on their own target and not on the targets of the wild-type plasmid or of the remaining mutants. The only exceptions are *svir2* and *svir7*, which seem to have the same specificity. Our sequence data identify them as identical.

We conclude from these experiments that a mutation in the target of this repression system causes a complementary alteration of the inhibitor itself so that the control mechanism remains functional despite the change in specificity. These properties are consistent with a repression mechanism based on base pairing between complementary nucleic acid.

#### svir mutants define new compatibility groups

The 'inhibitor dilution' model<sup>9</sup> proposes that incompatibility is the result of the inhibitory activity of a negative regulator of plasmid replication that is unable to distinguish between two homologous replicons. Given the results of the specificity test in Fig. 2, this model predicts that each *svir* mutant should be compatible with all the others but incompatible with itself. To test this prediction we constructed (Fig. 3) tetracycline-resistant derivatives of the wild-type plasmid and of the three mutants with different specificities (*svir7*, *svir11*, *svir12*). Heterozygous cells containing all the possible combinations of ampicillin- and tetracycline-resistant derivatives were constructed by co-transformation of the *recA* strain HB101. The percentage of clones

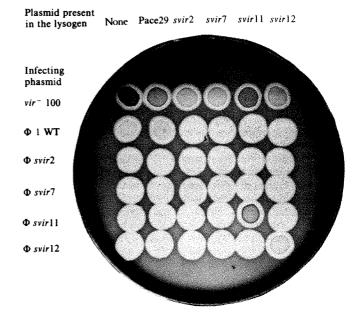


Fig. 2 Specificity test. The plate was prepared by overlaying a BBL plate (Baltimore Biological Laboratories; trypticase  $10 \, \mathrm{g} \, \Gamma^{-1}$ ) with 3 ml of top agar (as BBL agar but only 6.5 g agar per 1) containing 0.2 ml of a saturated culture of GC57 =  $\Delta(lac-pro)malA$  metal arg  $E_{am}$  rif thi supf. This strain is used as a permissive strain for all the phasmids tested. 0.3 ml of a dilution of phasmid lysates containing  $\sim 10^7$  plaque-forming units per ml was added to the wells of the horizontal lines of a microtitre plate. The wells in the vertical lines of the resulting grid were further inoculated with 0.05 ml of the lysogenic strain Q37 (thr leu lacY supE ( $\lambda P_{am3}$ )) containing the plasmid pacl29 or one of its svir derivatives. 5  $\mu$ l of the content of each well were spotted in a corresponding grid on the plate containing GC57. The photograph was taken after 24 h incubation at 37 °C. vir100 is a replication-defective derivative of the phasmid  $\Phi$ 1 (ref. 8). WT, wild type.

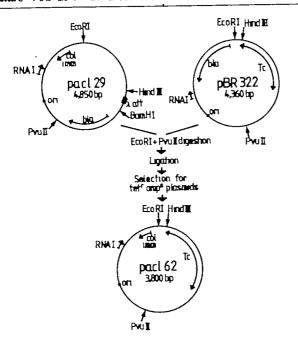


Fig. 3 Incompatibility properties of sour derivatives. Tetracycline-resistant derivatives of the plasmid paci29 and of the three sour mutants with different specificities, were constructed using the strategy shown. DNA from the plasmid pBR322<sup>14</sup> and paci29 was digested with the restriction endonucleases EcoRI and PosII. Equimolar amounts of the two digested DNAs were mixed at a concentration of 10 µg mi<sup>-1</sup> and ligated overnight at 14 °C. After transformation of a culture of HB101 made competent by CaCl<sub>2</sub> treatment, the transformed clones were selected on tetracycline plates (10 µg mi<sup>-1</sup>). The desired paci62 structure can be recognized from reconstituted pBR322 by replica plating on ampiculih plates (100 µg mi<sup>-1</sup>) sou? and source pacific derivatives have lost the PosII site in this construction probably because of some exonuclease activity in the enzyme preparation

that had lost either of the two plasmids after growth in non-selective medium was monitored approximately every 20 generations by plating in the presence of antibiotics. Table 1 shows the number of generations required to decrease by one order of magnitude  $(D_{-1})$  the number of clones containing both plasmids. As predicted by the inhibitor dilution model, only combinations that have cross-reacting inhibitors result in rapid segregation of clones containing only one of the two plasmids. After 150 generations, less than 1 out of 200 clones had lost one of the two plasmids in the segregation experiments on either side of the diagonal of Table 1.

These compatibility experiments support the hypothesis raised in the previous section and prove that, as predicted by the inhibitor dilution model, an alteration of the specificity of the inhibitor—target interaction results in altered compatibility properties. Given the peculiar repression system devised by ColE1-type replicons, it is possible to create new compatibility

Table 1  $D_{-1}$  values in segregation experiments

Rolativo	Ampicilin- resstant	<u> </u>			
copy number	plasmid	paci62	sver7	soir11	svir12
1	pac129	40-50	<b>≯</b> 150	>150	<b>≯</b> 150
3.5	sou?	<b>≫150</b>	40-50	<b>≯150</b>	<b>≯150</b>
0.7	sver11	<b>≯150</b>	<b>≯</b> 150	6070	<b>≫</b> 150
1.5	sour12	<b>≯</b> 150	<b>≯150</b>	<b>≫15</b> 0	50-60

 $D_{-1}$  values are given for the segregation experiment of bacterial strains containing the various combinations of ampicillin- and tetracycline-resistant derivatives. Clones containing two plasmids were constructed by co-transformation and selection on plates containing both antibiotics. One doubly resistant clone for each transformation was plated on an L plate and one colony still resistant to both antibiotics was grown for several generations (with dilutions in the plateau phase) in non-selective conditions. Every 20 generations the cultures were intrated and one plate containing ~200 colomes was replica-plated on to ampicillin and tetracycline plates. The number of generations required to decrease by a factor of 10 the percentage of cells containing both plasmids  $(D_{-1}$  values) were obtained from such segregation curves. The values in the diagonal are the average of three independent determinations. The first column shows the relative copy numbers of some mutantial.

groups by single base pair changes. Our results are not consistent with models of the type proposed by Bedbrook *et al.*<sup>10</sup> that stress the importance of membrane replication–segregation sites in determining plasmid incompatibilty.

The  $D_{-1}$  values of the different *svir* mutants were not proportional to plasmid copy number. We cannot explain this result, which seems to conflict with models based on random replication and random segragation<sup>11,12</sup>.

#### Location of svir mutants

The four base pair changes that cause the change in specificity of the control mechanism have been previously localized by in vivo deletion mapping between nucleotides 295 and 511 upstream from the origin of plasmid replication. This DNA fragment was sequenced by the chemical degradation method and revealed in each case a single base pair transition in the region that codes for RNA I. Figure 4 shows the base changes

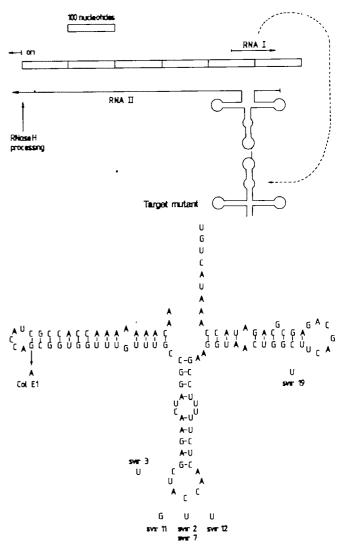


Fig. 4 Model of the ministory interaction between RNA I and the primer precursor and DNA sequence of the mutants. The DNA sequence of the wild-type pard29 (no difference from the published pBR322 sequence <sup>13</sup>) and of the sour mutants was obtained using the chemical degradation method <sup>13</sup> end labelling with DNA polymerase (Klenow subunit). The wild type and the two mutants obtained with nitroeoguanidine mutagenesis (rol2, sou7) were sequenced from the PoulI site ~500 nucleotides downstream from the origin of replication up to the end of RNA I in order to be able to exclude the possibility of secondary mutations, sou?11 and sou?12 (obtained by mutD mutagenesis) were sequenced only in the HpaII fragment that was shown by deletion mapping to contain the mutation, sou?3 and sou?19 cannot be propagated in the plasmid form. The Sau3A-TaqI DNA fragment that was used for sequencing was therefore prepared from phasmid DNA. The arrow in the right loop of the RNA I synthesized by the related plasmid CoIE1 The row mutations are shown as alterations of the RNA sequence of the primer precursor.

with respect to the wild-type sequence. The three base transitions at positions 55, 56 and 57 of the RNA I sequence indicate the importance of this triplet in the nucleic acid-nucleic acid interaction controlling pMB1 replication. The number of mutations isolated and sequenced is insufficient to define the limits of the site of interaction between repressor and target. Nevertheless, the clustering of the four mutations at the top of the central loop of the cloverleaf structure of RNA I is reminiscent of a 'codon-anticodon type' interaction between transfer RNA and messenger RNA. We do not know whether this observation has any functional or evolutionary significance.

# Target mutants that alter the cloverleaf

The two remaining inhibitor-insensitive mutants, svir3 and svir19, when released from the  $\lambda$  chromosome, are lethal for the host cell. We assume that this phenotype is due to uncontrolled replication. Strong selection for plasmid release and maintenance results in deletions of part of the mutant plasmid. The two mutations were localized by deletion mapping between nucleotides 295 and 511 from the origin of DNA replication. The corresponding DNA fragment was prepared from phasmid DNA and sequenced by the chemical degradation method. svir3 was found to be a base substitution that might affect target function by extending the central stem of the cloverleaf structure, thus preventing the anticodon-type base pairing with the inhibitor. The tRNA-like secondary structure is also disturbed by the  $C \rightarrow T$  transition in svir19 that affects the stability of one of its stems.

#### **Conclusions**

In four of the svir mutants that have been characterized, single base pair transitions resulted in a change of specificity in the mechanism of control of copy number without destroying its function. We believe this to be the first description of such a mechanism, which allows evolution while ensuring protection of function. It is possible that the relevance of this mechanism extends to biological phenomena in which self recognition plays

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a role. A single mutation may allow for the evolution of a new class of specificity while preserving, through the complementary alteration of the target sequence, the mechanism of self recognition.

Our results point to the crucial function of the central loop of RNA I in the interaction that leads to inhibition of initiation of ColE1 replication. Conclusions about the bases involved in these interactions must await the isolation and characterization of more mutants of different specificities. The specificity test that we have devised (Fig. 2) offers an easy tool for this type of

It is interesting that the mutant copy number phenotype is correlated with the free energy content of the hydrogen bonds between the nucleotides of the two triplets postulated to be involved in the interaction.  $C \rightarrow U$  transitions respond to increase in copy number while the only  $A \rightarrow G$  transition results in a low copy number plasmid, probably as a consequence of the better repressor target interaction. The dramatic change in the efficiency and specificity of the control mechanism caused by single base substitutions suggests that this interaction does not involve a large number of bases.

From our results and the in vitro experiments of Tomizawa et al.6, we propose (Fig. 4) that the base pairing between the central loop of RNA I and the complementary structure on the nascent primer precursor is essential in preventing maturation of this latter transcript, probably by preventing the formation of the RNA-DNA hybrid that is the substrate for the maturation enzyme RNase H. Furthermore, we have evidence that the stability of the cloverleaf structure of the target is a requirement for successful inhibitory interaction. Two mutations that alter two of the stems in the target putative secondary structure result in insensitivity to wild-type inhibitor. Our results, however, do not allow us to distinguish whether RNA I interacts with the primer precursor or with its own DNA coding sequence. This will require further genetic and biochemical experiments.

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# Two conserved sequence blocks within eukaryotic tRNA genes are major promoter elements

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The split promoter sequences of a tRNA<sub>CUG</sub> gene of Xenopus laevis have been mapped to nucleotides 13-20 and 51-64 of the tRNA Leu coding sequences. The sequences closely coincide with two conserved sequence blocks present in all eukaryotic tRNA genes. The two conserved sequence blocks were found to be exchangeable between tRNA genes as chimaeric tRNA det et RNA genes proved transcriptionally active. Furthermore, two prokaryotic tRNA genes exhibiting strong homologies with the two blocks yielded specific transcripts when tested in an eukaryotic transcriptional system.

THE tRNA Met genes of Xenopus laevis have an unusual splitpromoter arrangement in that sequences directing transcription are located near both the 5' and the 3' end of the tRNA Met gene unit1. As in the case of the 5S RNA gene2.3 of the same genus and the VAI gene of adenovirus4, the control regions of the tRNA<sup>Met</sup> are located within the structural gene itself (reviewed in ref. 5). Here, we show that a tRNA CUG gene of X. laevis has a similar split promoter and trace the essential sequences to two

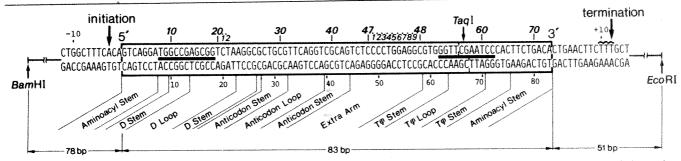


Fig. 1 DNA sequence of the tRNA Lew gene of X. laevis. The DNA sequence coding for the tRNA Lew is boxed. The numbers in bold-faced type above the box are in agreement with the standardized numbering system? Consecutive numbering is shown below the box. The bars between the two DNA strands show the location of the conserved elements which are discussed in detail in Fig. 4 legend.

sequence blocks which are highly conserved in all eukaryotic tRNAs. We present evidence that the promoter organization of genes transcribed by polymerase III can differ substantially and argue that the split promoter arrangement of the tRNA genes is in keeping with the evolution and functional diversity of the tRNA structure.

#### The tRNA<sup>Leu</sup> gene transcription unit

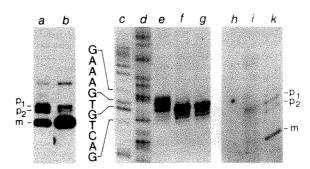
Figure 1 displays the DNA sequence of the X. laevis tRNA<sub>CUG</sub> gene<sup>6</sup> used in the present study, together with the DNA sequences of the adjacent flanking regions. The numbers in bold-faced type above the DNA sequence of the structural gene are in accord with the standard tRNA numbering system<sup>7</sup>, which is based on the primary structure of the yeast tRNA<sup>Phe</sup> and is used here throughout. The tDNA Leu fragment was isolated from a cloned 3.18-kilobase (kb) DNA repeat<sup>8</sup> by cleavage with Mbol and EcoRI and was then cloned between the EcoRI and BamHI sites of pBR322. On injection of the recombinant plasmid with  $[\alpha^{-32}P]$ GTP into the nuclei of frog (Xenopus laevis) oocytes<sup>9</sup> (for technical details see ref. 10) followed by a short incubation period of 3 h, three labelled RNAs (m, p<sub>1</sub> and p<sub>2</sub>) were detected by electrophoresis on denaturing polyacrylamide gels (Fig. 2, lanes a, b). Band m was previously identified by RNA sequencing as mature tRNA<sup>Leu</sup> (S. Clarkson and R. Koski, personal communication) containing 85 bases, including the common 3'-terminal CCA sequence which is added post-transcriptionally. The two larger RNAs p<sub>1</sub> and p<sub>2</sub> are 98 and 95 nucleotides long, respectively, and represent precursors to the mature tRNA<sup>Leu</sup>. The intensity of band m increased with longer incubation times whereas bands p<sub>1</sub> and p<sub>2</sub> were only faintly visible after overnight incubation.

The approximate position of the 5' termini of p<sub>1</sub> and p<sub>2</sub> on the tDNA was determined by S<sub>1</sub> mapping experiments<sup>5,11</sup> using individual, gel-purified p<sub>1</sub> and p<sub>2</sub> RNA and a complementary hybridization probe, 5'-end labelled at the TaqI site (see Fig. 1). After S<sub>1</sub> digestion of the hybrids, the products were fractionated on a denaturing polyacrylamide gel: p2 and m gave identical S<sub>1</sub>-protected DNA bands (Fig. 2, lanes f, g) and hence these molecules are presumed to have identical 5' termini; therefore, both molecules have a G as the first base in the sequence. The DNA fragments protected by  $p_1$  were two or three bases longer, hence the 5' end of  $p_1$  maps at the A or C at position -3 or -2. We next investigated whether both p<sub>1</sub> and p<sub>2</sub> could be primary transcripts. [\(\beta^{-32}\)P]GTP or -ATP were injected with the tRNALeu gene and RNA was isolated after a short 30-min incubation to avoid transfer of the label to the  $\alpha$  position<sup>5</sup>. Injection with  $[\beta^{-32}P]GTP$  did not result in labelling of any of the transcripts, whereas injection of  $[\beta^{-32}P]ATP$  labelled only the  $p_1$  transcript (Fig. 2i). We conclude that  $p_1$  is a primary transcript commencing with an A at position -3 and that p<sub>2</sub> and m are derived from p<sub>1</sub> by processing at the 5' end. Considering the length of p<sub>1</sub> and p<sub>2</sub>, it seems likely that the 3' end of both precursors maps to position +11 or +12, that is, to the two last bases of the putative termination signal TTCTTT in the 3'flanking sequence.

#### The intragenic promoter

Having established the size of the tRNA<sup>Leu</sup> transcription unit, we constructed deletion mutants lacking sequences at either the 5' or the 3' end of the tRNA<sup>Leu</sup> gene. The BamHI and EcoRI sites at positions -78 and +51, respectively, provided convenient starting points for resection with exonuclease Bal31. After the addition of HindIII linkers to the resected ends, the truncated tDNA<sup>Leu</sup> fragments were recloned, all in the same orientation, into the pBR322 vector, between either the EcoRI and HindIII or the BamHI and HindIII sites. The extent of the sequence truncation was determined by DNA sequencing<sup>12</sup> and its effects on transcription finally assessed by oocyte injection. To normalize the amount of transcription in each injection experiment, cloned 5S DNA of X. laevis<sup>13</sup> was routinely coinjected as an internal control at a level of one 5S RNA gene per four tRNA<sup>Leu</sup> genes.

The resected clone a (Fig. 3), which lacks most of the 5'-flanking region but still contains the precursor transcription unit, is transcribed at the same level as the progenitor clone. Further



Analysis of transcripts made from the tRNA wild-type gene. The small DNA fragment shown in Fig. 1 was isolated from a cloned 3.18-kb DNA repeat unit8 after cleavage with EcoRI and Sau3A and was then cloned with pBR322 between the EcoRI and the BamHI restriction sites. Plasmid DNA was isolated by the clear-lysate method<sup>22</sup> and further purified by isopropanol precipitation and centrifugation in a CsCl step gradient. After recovery and reprecipitation, the DNA used for oocyte injection was dissolved in 88 mM NaCl, 10 mM Tris HCl pH 7.4, at a concentration of 200  $\mu$ g ml<sup>-1</sup>. A 20 nl aliquot of this DNA solution containing  $0.2 \,\mu$ Ci of  $[\alpha^{-32}P]$ - or  $0.4 \,\mu$ Ci of  $[\beta^{-32}P]$ -triphosphates was injected into nuclei of centrifuged oocytes as described earlier<sup>9,10</sup>. The oocytes were incubated for 0.5-16 h and total nucleic acids then isolated. The labelled RNAs were analysed by electrophoresis through 10% polyacrylamide, 8 M urea sequencing gels and detected by exposure to X-ray film using an intensifier screen. For S<sub>1</sub> mapping, individual RNAs (m, p<sub>1</sub> and p<sub>2</sub>) were isolated from the gel<sup>12</sup> and the RNA from one oocyte was hybridized to the 5' half of a tDNA<sup>Leu</sup> fragment which had been 5'-end labelled at the *Taq*I site (compare Fig. 1). Hybridization was carried out in 20 µl of 80% formamide, 0.4 M NaCl, 40 mM MOPS pH 6.7, and 1 mM EDTA at 51 °C for 16-24 h. The reaction was stopped on ice with 180  $\mu$ l ice-cold S<sub>1</sub> buffer (30 mM NaAC pH 4.5, 2 mM ZnSO<sub>4</sub>, 0.2 M NaCl). 10 U of S<sub>1</sub> enzyme were added and the samples were incubated at 41 °C for 45 min. The analysis of the protected DNA fragments was carried out on a 10% polyacrylamide, 7 M urea sequencing gel. Lanes a, b, RNA isolated from two oocytes incubated for 3 (a) and 15 (b) h; lanes  $e, f, g, S_1$  mapping of  $p_1(e), p_2(f)$  and m(g); lanes c, d, A-specific (d) and G-specific (c) sequence reaction of the DNA probe used for  $S_1$  mapping; lanes h, i and k, labelling with  $[\beta^{-32}P]GTP$  (h) and  $[\beta^{-32}P]ATP$  (i) and  $[\alpha^{-32}P]GTP$  (k) for 0.5 h. RNAs extracted from 46 (h), 52 (i) and 1.5 (k) oocytes were loaded onto the gel. Exposure time was 48 h.

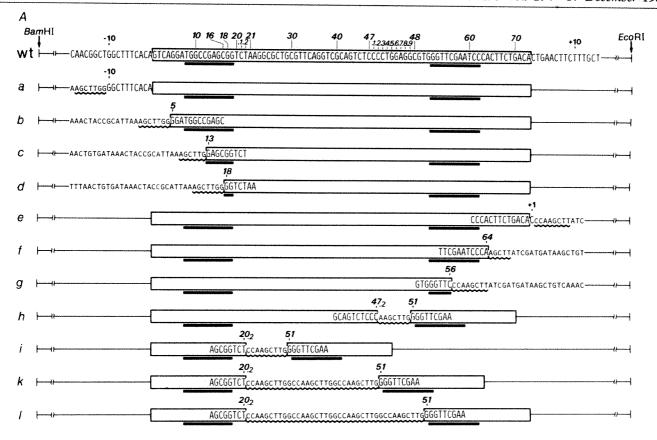


Fig. 3 Structure and transcription of the tDNA<sup>Leu</sup> mutants. The starting material for the resection of the tRNA<sup>Leu</sup> gene was the clone described in Fig. 2 legend. 15 µg of this plasmid were cleaved with EcoRI and another 15 µg with BamHI. The linearized DNA was treated for 30 min at 30 °C with 0.2 U Bal31 double-strand exonuclease<sup>23</sup> in 20 mM Tris pH 8.1, 600 mM NaCl, 12 mM MgCl<sub>2</sub>, 1 mM EDTA. In these conditions up to 200 nucleotides are resected from each end. 40 pmol of synthetic *HindIII* linker labelled with  $[\gamma^{-32}P]$ ATP were ligated to the *Bal31*-digested DNA, giving about a 15-fold molar excess of linker to DNA ends. Unreacted HindIII linkers were removed by centrifugation through a 5-20% sucrose gradient using a SW40 rotor. The plasmid DNA was precipitated and after resuspension in buffer digested with 80 U HindIII followed by BamHI or EcoRI. The DNA fragments were separated on a preparative 8% polyacrylamide gel and eight gel slices were cut out containing DNA fragments ranging from 50 to 200 bp. After elution from the gel and chromatography on DEAE-Sephadex, the DNA fragments were cloned with pBR322 which had been previously cleaved with EcoRI and HindIII or BamHI and HindIII and then treated with phosphatase24. Recombinant plasmids were identified by restriction analysis and DNA sequencing. To produce insertion and deletion mutants, the individually cloned small subfragments were prepared from the recombinant plasmids by digestion with EcoRI and HindIII or BamHI and HindIII followed by preparative polyacrylamide gel electrophoresis, elution from the gel and chromatography on DEAE-Sephadex. 1 pmol of pBR322

B M wt a b c d e f g h i k / 309 240 201 180 160 147 122 110 90 76 67

plasmid DNA which had been cleaved with EcoRI and BamHI and treated with alkaline phosphatase was ligated with 1 pmol each of two corresponding DNA fragments. A, structure of the different tDNA<sup>Leu</sup> mutants. The structural gene is boxed, X. laevis DNA sequences are shown in large letters, HindIII linkers and pBR322 sequences in small letters. HindIII linkers are underlined with wavy lines. Original sequences at the junctions which have been restored by the addition of HindIII linkers were considered as part of the gene unit. The bars below the sequence indicate the conserved sequence motifs. B, transcriptional analysis of the mutants carried out as described in Fig. 2 legend. Incubation time after injection was 3 h for wt and mutants a, e and h, and 15 h for mutants b, c, d, f, g, i, k and l. RNA from two oocytes was loaded on to the gel and the film was exposed overnight. Lane M, pBR322 plasmid DNA digested with HpaII and end labelled with  $[\gamma-^{32}P]ATP$ . The length in nucleotides of the single-stranded DNA fragments is given to the left.

resection up to and including nucleotide 4 of the structural gene (clone b) deletes the normal initiation site but permits continued synthesis of several precursor molecules, albeit at a slightly reduced rate.  $S_1$  mapping demonstrated that the majority of the transcripts from clone b initiate at the junction between plasmid and linker DNA and their heterogeneous length seems to stem from inefficient termination and incorrect processing (data not shown). The 5' border of essential promoter sequences can be placed around position 13, as clone c, which lacks 12 nucleotides of the structural gene, is still transcribed, although very poorly, whereas clone d, which lacks 16 nucleotides, is totally inactive.

Removal of the 3'-flanking sequences, including the putative termination signal TTCTTT(clone e), did not affect the rate of transcription but led to the synthesis of a 165-base precursor which, as deduced from its length, terminated within a run of Ts

located in the pBR322 plasmid DNA at positions 4,323–4,327 (ref. 14). A mutant which lacked 9 base pairs (bp) of the 3'-terminal sequences of the structural gene was transcribed at a reduced rate (clone f). However, a mutant lacking 17 bp was completely inactive (clone g). From the results of these two sets of experiments we conclude that the essential promoter sequences of the tRNA<sup>Leu</sup> gene, detectable by oocyte injection experiments, are located somewhere between positions 13 and 64 of the structural gene.

#### The split promoter of the tRNA Leu gene

To assess whether the gene-internal tDNA<sup>Leu</sup> promoter is discontinuous, as is the case for the tDNA<sup>Met</sup> promoter, we constructed mutants in which the central portion was replaced by a variable number of *HindIII* linkers. In one such tDNA<sup>Leu</sup>

mutant the 14-bp region coding primarily for the extra arm of the tRNA Law (standardized positions 47.3 and 50) was replaced by a 10-bp synthetic HindIII linker DNA segment. This mutant was transcribed almost as efficiently as the wild-type gene (Fig. 3, clone h). Another mutant in which the 16 bp at positions 21-37 were replaced by 30 bp provided by three concatenated HindIII linkers, yielding an expanded gene, resulted in about 40% of the normal level (data not shown). In a third mutant, a segment 40 nucleotides long (positions 21-50) was replaced by one, three or four HindIII DNA linkers. The mutant containing one *HindIII* linker proved inactive in transcription (clone i). Substitution of the deleted sequences by three HindIII linkers reactivated the gene unit to a very small extent (clone k). Four HindIII linkers, which together exactly replace the length of the deleted sequences (clone I), activated transcription to a moderate level (discussed below).

Our previous experiments with the tRNA<sup>Mot</sup> gene<sup>5</sup> showed that control sequences were located in the anterior and posterior portions of the structural gene. Considering the above substitution-deletion mutants of the tRNA<sup>Lon</sup> gene as well as those created by 5' and 3' resection, a picture emerges which is more differentiated than that provided by the previous tDNA mutants. All four boundaries of the two gene-internal promoter

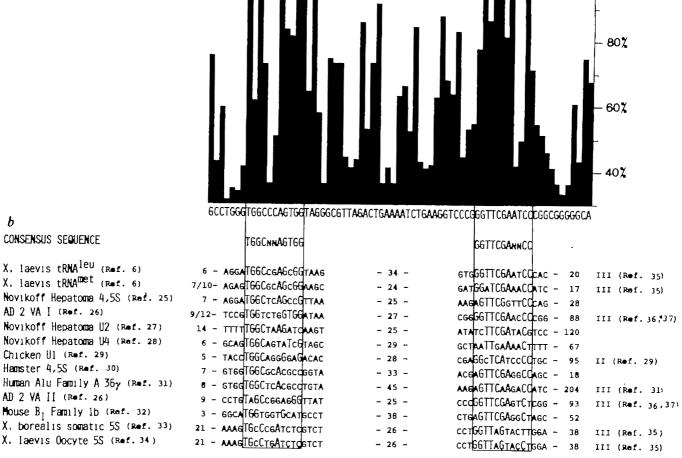
blocks have now been determined with some precision. By comparing the autoradiographic signals of the tRNA transcripts with that of the 5S RNA control, a rough estimate of the differences in tRNA production by the various injected clones can be made. Resection of the tRNA gene to position 5 is found to reduce transcription to a small degree (but curiously the product is now polydisperse and transcription termination is affected), whereas removal of sequences up to nucleotide 13 reduces transcription by a factor of about 20. Transcription is abolished altogether when the sequences up to and including nucleotide 16 are removed. This is in agreement with the tRNA mutants, where resection up to nucleotide 8 had little effect but resection up to nucleotide 12 reduced transcription to below the levels of detection.

Deletion of all 3' sequences, including the termination signal, up to +1 of the sequences located 3' to the structural gene, nevertheless permits transcription at a level of 70% of control. When the terminal 9 bp of the structural gene are removed up to and including position 65, transcription continues, albeit at a fourfold reduced level, but deletion of the next eight nucleotides completely abolishes transcriptional activity. The results therefore suggest that transcription is reduced as resection of sequences approaches the two gene-internal conserved

B

ю

100%



30

40

60

Fig. 4 Conserved sequence blocks A and B. a, The profile shows the frequency of occurrence of the most common nucleotide at every position in eukaryotic tRNAs as derived from a compilation of 80 eukaryotic tRNA's equences? The sequence of the most frequent nucleotides is given below the profile. The arrows (α, β, γ) above the profile indicate discontinuities in the compiled tRNA sequences. α, Nucleotide 17 is omitted because it is only present in 40% of the sequence tRNAs. β, Nucleotides 20 and 21 are separated by a variable number of nucleotides; thus, nucleotide 21 is not included in the A block γ, Location of extra arm. The consensus sequence for the A and B block includes all nucleotides which occur with a frequency of over 60%. Sequences of selected small cellular RNAs were taken from the references given on the left. They were aligned for maximal homology to the A and B block region. Nucleotides matching with the consensus sequence are shown with large letters. The numbers given on the left and on the right of each sequence represent the numbers of nucleotides to the initiation and termination points, respectively. The numbers in the middle show the number of omitted nucleotides. The roman numbers to the right of several sequences indicate which of the RNA polymerases transcribes the gene.

sequence blocks (underlined in Fig. 3; see below) but falls off precipitously when sections of these sequence blocks are removed. The internal sequences 21–37, as well as 47.3–50 can be replaced by *HindIII* linker and still support transcription of at least 40% of the wild-type level. This may be compared with the related tRNA<sup>met</sup> mutants, where substitutions of nucleotides 30–50 by *HindIII* linkers still permitted tRNA transcription, although at a reduced rate. These experiments show that gene sequences 21–50 can be replaced by foreign DNA, thus delimiting the gene-internal boundaries of most essential sequences. Note, however, that substitution of the entire sequence from 21 to 50 by *HindIII* linkers strongly inhibits transcription.

Thus, deletion and substitution of DNA segments around nucleotides 10–20 and 51–64 produces the most pronounced inhibition of transcription. Substitutions of small segments between these sequence blocks have smaller but nevertheless deleterious effects on transcription and these effects are apparently potentiated when several segments are removed together, as is the case for the 21–50 substitution. A picture therefore emerges for the tRNA<sup>Met</sup> and the tRNA<sup>Leu</sup> gene in which nucleotides particularly important for transcription promotion are seen to be clustered in two sequence blocks. Our unpublished findings that a minimal gene consisting entirely of DNA linkers and the above sequence blocks proved inactive in transcription indicate that these sequence blocks are necessary, but not sufficient, for initiation of tDNA transcription.

#### Conserved sequences in eukaryotic tRNAs

Figure 4a displays a profile showing the frequency of occurrence of the most common nucleotides at every position in eukaryotic tRNAs, as derived from a recent compilation of 80 tRNA sequences<sup>7</sup>. Two regions at positions 8-19 (A block) and 52-62 (B block) are highly conserved, with several nucleotides occurring at a frequency of 100%. The regions outside the A and B blocks exhibit a lower degree of nucleotide preference. Conservation of these sequences in both prokaryotic and eukaryotic tRNA sequences has previously been interpreted solely in terms of their importance to tRNA structure and hence protein synthesis; for instance, in the yeast tRNA Phe, positions 8, 9, 14, 15, 18, 19 and 54-56 are essential in stabilizing the tertiary structure of the tRNA molecule<sup>15</sup>. Our present work on the tRNA Leu gene has demonstrated that virtually all the nucleotides which are external to the conserved A and B blocks can be removed without eliminating transcription, while conversely, deletion of the conserved sequences abolishes transcription altogether. Hence, nucleotides in the A and B block have not only a function in tRNA structure but also in tRNA gene transcription.

If the conserved A and B blocks are the principal, functionally distinct promoter sequences, these blocks should be exchangeable between different tRNA genes without affecting their transcriptional activity. To test this idea we constructed two chimaeric genes which contained the 5' half of one and the 3' half of the other from the tRNA<sup>Met</sup> and the tRNA<sup>Leu</sup> structural genes and flanking sequences. Both chimaeric genes (Fig. 5, lanes 3, 4) were transcriptionally active in the frog oocyte. This focuses our attention again on the sequences held in common between these two tRNA genes and further suggests that the self complementarity of the acceptor stem sequences of tRNA genes is apparently not an important aspect of the tDNA promoter structure.

Another prediction of our model is that prokaryotic tRNA genes exhibiting strong A and B homologies should yield specific transcripts when tested in an eukaryotic transcriptional system. This was found to be the case for the tRNA<sup>Asp</sup> and the tRNA<sup>Trp</sup> of Escherichia coli (W. Folk, unpublished data). Conversely, the E. coli tRNA<sup>Tyr</sup> gene is not transcribed when injected into frog oocytes (cited in ref. 16), and this prokaryotic gene shows poor homology to the A block sequence. All these results strengthen our hypothesis that the two conserved homology blocks are important for the transcription of eukaryotic tRNA genes.

### Diversity of promoters for polymerase III

If we consider all bases occurring at a frequency of at least 60%within sequence blocks A (positions 8-19) and B (positions 52-62) in over 80 different eukaryotic tRNAs, a 'con-TGGCNNAGTGG (A block) sequence' GGTTCGANNCC (B block) can be abstracted. Note that in some tRNA genes one or two additional nucleotides interrupt the A block at position 17. Therefore, the last two Gs of the A block either do not need to be co-linear with other nucleotides or are dispensable for transcription in some tRNA genes. Fowlkes and Shenk reported that two such sequence blocks also occurred in the adenovirus VAI and VAII gene and the 4.5S RNA gene of hamster cells. After mapping essential promoter sequences somewhere between positions 9 and 72 in the VAI gene4 they speculated that this sequence motif may be involved in transcription of RNA polymerase III genes. This hypothesis has now been shown to be correct for the  $tRNA^{Leu}$  gene of X. laevis.

The A and B sequence homologies can be extended to other genes transcribed by RNA polymerase III, for instance, to members of the human AluI and the mouse B1 families (Fig. 4b). Surprisingly, similar conserved blocks are also found in some of the genes coding for the U-RNA series, which code for capped RNAs and are therefore generally assumed to be transcribed by RNA polymerase II. The B block homology is particularly striking for many of these genes and it would therefore be worthwhile to investigate the potential of these genes to be transcribed by RNA polymerase III. Note that for most of the genes listed transcription initiation occurs 10-13 bp 5' to the A block, and in all cases (except for the 5S RNA genes) within 7-16 bp 5' to the A block boundary.

In Fig. 4b the genes have been arranged in order of diminishing strength of A block homology. This places the X. laevis oocyte 5S RNA gene clearly at the bottom. It is doubtful whether the A block homology of the 5S RNA is actually statistically significant. Dissection experiments have shown the 5' portion of the gene to be largely dispensable in transcription promotion. However, more recent competition experiments also implicate the whole area from -11 to 28 (rather than a specific A block segment) in some transcriptional role 17. The internal control region of the 5S RNA gene positions 50-83, although containing the B block homology, is considerably

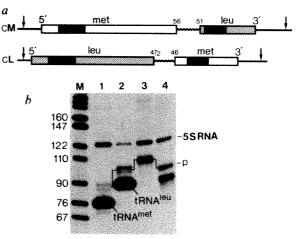


Fig. 5 Transcription of chimaeric tRNA genes. a, The chimaeric gene cM was constructed by combining, via HindIII linkers, the 3' half of mutant h (Fig. 3A) of the tRNA Leu gene with a tDNA Met fragment containing 120 bp of 3'-flanking sequences and the first 56 nucleotides of the structural gene 5. Lis a combination of the 5' part of mutant h (Fig. 3A) of the tRNA Leu gene with a tDNA Met fragment which contains 28 bp of the 3' end of the structural gene and stretches 90 bp into the 3'-flanking sequences<sup>5</sup>. Wavy lines represent HindIII linker DNA, black boxes show the location of the conserved sequence blocks, arrows at the 5' end and 3' end indicate initiation and termination points, respectively. b, Lanes 1-4, transcriptional analysis of wt and mutant genes. Incubation time after injection was 15 h. RNA from two oocytes was loaded on to the gel and the film was exposed overnight. Lane 1, tRNA Met wt; lane 2, tRNA Leu wt; lane 3, chimaeric gene cM; lane 4, chimaeric gene cL.

larger than the posterior control region of tRNA genes. Therefore, both the primary structure of the 5S RNA and the tRNA genes and their response to sequence manipulation suggest that there are at least two subclasses of RNA polymerase III promoters (see also below) which differ from one another in their arrangement and the relative importance of specific sequence blocks. Other genes known to be transcribed by RNA polymerase III seem to fall between these two extreme cases. The above grouping of the genes is further supported by the results obtained from cofactor separation. Faithful transcription of the *Xenopus* tRNA<sup>Met</sup> and the adenovirus VAI gene depends on similar protein fractions<sup>18</sup>, whereas the 5S transcription requires an additional cofactor which binds to the intragenic control region 50-83<sup>19,20</sup>.

From previous experiments with the tRNA<sup>Met</sup> gene unit<sup>5</sup> it can be calculated that the minimal distance between the A and B blocks (see Fig. 4b) is about 29 bp. The tRNA<sup>Met</sup> gene is one of the smallest genes transcribed by RNA polymerase III and in this gene the A and B block probably lie together in the closest proximity compatible with efficient promotion of transcription. However, the distance A-B in natural genes can be much larger, for instance, 41 bp in the Xenopus tRNA Leu gene or 68 bp in the

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yeast tRNA<sup>Leu</sup> gene, which contains an intron<sup>21</sup>. It is therefore not surprising that the RNA polymerase III gene system can cope with tRNA<sup>Met</sup> genes distended by 30 bp<sup>1.5</sup> or with introns of the tRNA<sup>Leu</sup> gene of yeast, into which additional sequences have been inserted<sup>21</sup>. The intermediate coding regions of tRNA genes located between the conserved A and B blocks include the sequences coding for the anticodon loop, the extra arm of different length (see Fig. 1) and the insertion sites of naturally occurring introns. It is therefore this region that predominantly differs between the various tRNA molecules. In keeping with this, the split tRNA promoter permits variability not only from single base changes, but also from extension or shortening of the sequences lying between the A and the B block. This flexibility contrasts with the 5S RNA gene operator system where only a single major type of RNA is produced and where transcription initiation occurs at a fixed distance from a single gene-internal control sequence

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# Human immunoglobulin D segments encoded in tandem multigenic families

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A family of germ-line immunoglobulin D-region genes has been cloned and mapped at regular intervals along a 33-kilobase length of human chromosomal DNA. Each member of the family varies slightly in sequence, but precisely conserves the recombinational signals and spacing that flank each gene. This region seems to have been formed by the tandem amplification of large and still well conserved segments of genomic DNA. Further, structural comparisons of germ-line and rearranged D segments suggest that D segments may recombine with each other.

THE genetic information necessary to form an immunoglobulin molecule is encoded in separate segments of DNA that must be brought together to form a coherent gene 1-8. This process occurs in developing B lymphocytes<sup>9-11</sup> and is a central mechanism necessary for the creation of antibody diversity. Mouse immunoglobulin  $\kappa$ -chain genes, for example, are formed by joining one of several hundred germ-line V-region segments <sup>12,13</sup> to one of four active J regions <sup>14,15</sup>. These segments can be joined in different combinations and at slightly different cross-over points so that there are thousands of different possible end products 14-16.

Such recombination is thought to be mediated by special signals encoded 3' to each germ-line V-region sequence and 5' to

each functional J-region sequence 6-8,14,15. These signals are complementary to one another and consist of a small palindromic heptamer separated by an approximately 12- or 23-base spacer from an A- or T-rich nanomer. Thus, the consensus signal sequence, 5'-CACAGTG-12 base spacer-ACAAAAACC-3', occurs 3' to each V, gene, while the consensus sequence, -GGTTTTTGT-23 base spacer-CACTGTG-3', occurs 5' to each J<sub>s</sub> sequence. The sequences of the spacer regions are not conserved but their lengths apparently are. The conservation of these elements prompted the formulation of three rules for V-J recombination: the two signal sequences, each consisting of a nanomer and a heptamer part, must be complementary to each other<sup>14,15</sup>; recombination can occur only between a signal of 12

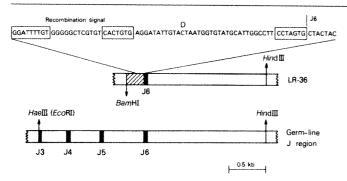


Fig. 1 Germ-line and aberrantly rearranged (LR-36) restriction fragments used for screening a Charon 28 phage library constructed from a partial MboI digest of human placental DNA. The novel DNA adjacent to J6 in LR-36 is depicted by cross-hatching; the nucleotide sequences comprising a D-like element are shown above. The LR-36 BamHI-HindIII fragment (1.9 kb) isolated from a pBR322 subclone was nick-translated to a specific activity of 200-500 c.p.m. per pg as described previously 32 and used to screen the phage library according to the Benton-Davis procedure 33. Fifteen hybridizing plaques were obtained and were rescreened with the embryonic HaeIII-HindIII fragment (~3 kb) in order to select those library clones that specifically hybridize to the novel germ-line D-like DNA shown by cross-hatching. (The embryonic HaeIII site was transformed into an EcoRI site by joining to the arm of the phage vector Charon 4A<sup>20</sup>.)

base pairs (bp) spacing with one of 23 bp spacing<sup>7,8</sup> and joining occurs near the ends of the heptamers, thus deleting the signal sequences in the recombinant<sup>14,15</sup>.

Formation of heavy-chain V-region genes follows these same basic principles but is somewhat more complex; it involves the joining of three separately encoded elements, a V and J sequence as well as an additional element that appears between them<sup>7.8</sup>. The additional segment, called D (for diversity), encodes a major portion of the third hypervariable region of heavy chains<sup>7.8.17</sup>. Because ~23-base spacers were found in the recombination signals that flank germ-line mouse  $V_H$  and  $J_H$  sequences, it was predicted that germ-line D segments would be flanked by recombination signals separated by ~12-bp spacers. Two initial examples in the mouse bear out this prediction<sup>18,19</sup>.

We set out to find and characterize such D-gene segments in man and to elucidate their organization and the extent of their representation in the human genome. The possibility of assembling V regions from three separate segments greatly increases the potential for generating diversity, but this potential immediately depends on the size of the germ-line repertoire of V, D and J region segments as well as the nature of the recombination event.

Our first requirement in any strategy designed to characterize germ-line D segments was to obtain a DNA probe containing germ-line D sequences so as to screen human genomic libraries. This posed a problem, because V-D-J recombinants generally contain only very short stretches of sequence derived from germ-line D segments and therefore are unsuitable as probes for screening. One way of circumventing this difficulty would be to isolate an intermediate in which the D segment had been joined to only one of its two eventual recombination partners, either the V or J segment. In this way, a longer segment of the germ-line sequence flanking the D segment would remain associated with the new V-D or D-J segment. We found such an intermediate, albeit somewhat aberrantly rearranged, in an IgM-bearing leukaemic lymphocyte in undergone rearrangement<sup>20</sup> chromosomes had chromosomal copy, the active one, had been produced by V-D-J recombination (LR-35); the other (LR-36) had been generated by joining a D-like segment to the 3'-most JH sequence, J6, to produce a D-J-type intermediate<sup>20</sup>. The 5 portion of this clone became our probe for a D-region family. We show below that this D segment is apparently encoded in a large although closely related family. Members of this family are widely but evenly spaced over a 33-kilobase (kb) segment of germ-line DNA, separated from one another by about 9-kb stretches of DNA. Surprisingly, these stretches are tightly conserved. The D segments vary slightly in sequence but retain their recombination signals. In addition, the structure of recombined D segments suggests that they might be shuffled, that is, recombine with each other, thereby further diversifying the third hypervarible region of heavy chains.

# D-gene segment has aberrantly recombined with a J region

Lymphocytes from patients with chronic lymphocytic leukaemia seem to represent the clonal expansion of a single differentiated cell. Both chromosomes of one such IgM-bearing leukaemic lymphocytic clone (CLL-LR) have rearranged with respect to the J<sub>H</sub> locus as discussed by Ravetch et al.<sup>20</sup>. One recombinant (LR-35) is presumed to be the active gene, giving rise to an expressed IgM molecule. The other recombinant (LR-36), however, has been generated by joining a D-like sequence with the 3'-most J<sub>H</sub> sequence, J6. This rearrangement is incomplete because V-gene sequences are lacking, and, furthermore, it is aberrant, because stop codons are located in phase with the J-coding frame. The new sequences brought in next to J6 include a recombination signal with a 12-bp spacer, as would be expected of such signals for the germ-like D segments 7.8,-8,-9 (see Fig. 1). Consequently, this aberrant rearrangement seems to represent a germ-line D-gene-like sequence joined to J6, thus providing us with a larger probe for germ-line D-gene segments.

To obtain germ-line D segments, we screened a human placental library constructed in the vector Ch28 by partial MboI

Fig. 2 Southern blot analysis of human placental DNA (a) and of fibroblast **(b)** DNA lymphocyte (c) from a leukaemic patient. Human **DNAs** were digested with the restriction enzyme BamHI, fractionated on a 0.8% agarose gel and then blotted on to nitrocellulose filters as described previously<sup>21</sup>. Filters were hybridized to the 1.8-kb germ-line BamHI fragment shown; this restriction fragment was derived from phage A3 shown in Fig. 3 and subcloned into pBR322. a, The probe hybridizes to itself (1.8-kb fragment) in addition to four more fragments 18, 7.4, 6.6 and 1.6 kb long. c. The germ-line D probe only hybridizes to those fragments located 5' to the D1 sequence which rearranged aberrantly with J6 (LR-36). Only the 18-kb and 6.6-kb fragments have been retained during DNA rearrangement in this B cell. (The rearranged fragment carrying the aberrant DJ6 recombinant (LR-36) is visible only after a long exposure as an about 12 kb band.) The fibroblast line (b)serves as a control and shows the same pattern seen in placental DNA

(a).

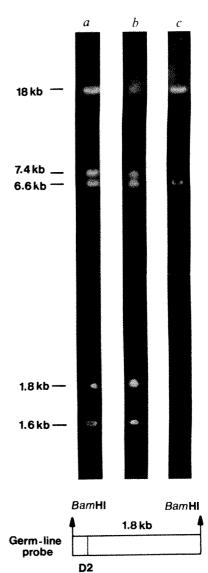
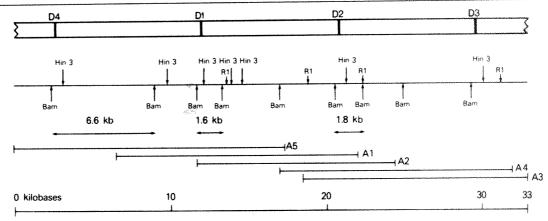


Fig. 3 Map of human linking four D locus separate D-gene seg-33 kb ments on genomic DNA. Shown are a partial restriction map, the approximate extent of phage clones used to construct this map and the three genomic BamHI fragments contained entirely on our clones (6.6, 1.8 and 1.6 kb long). D3 is located on a 7.4-kb genomic BamHI fragment (see which is only partially



contained on the phage A3. Phage clones A1-A5 overlap, whereas phage clone A6, containing only one D element (D5), has not been linked. The map illustrates the regular tandem arrangement of members of this family of D segments.

digestion. The fragment used as a probe is the 1.9-kb BamHI-HindIII fragment shown in Fig. 1, a D-J probe isolated from the aberrant recombinant LR-36. Fifteen hybridizing phage clones were isolated and then rescreened with an embryonic J probe (EcoRI-HindIII fragment in Fig. 1). Only those phage clones hybridizing to the D-J fragment but not to the embryonic J probe contain germ-line D-gene segments. In this way we obtained seven phage clones containing D-gene segments.

# D-gene segments exist as a family in germ-line DNA

To characterize the phage clones containing D-gene segments, the cloned DNAs were digested with BamHI and hybridized with the D-J probe (BamHI-HindIII in Fig. 1) according to the Southern blotting technique<sup>21</sup>. Such analyses revealed multiple BamHI fragments with D segments. A characteristic 1.8-kb D-segment-bearing BamHI fragment, found in four clones (A2, A3, A4 and A7), was subcloned into pBR322 and used as a germ-line D probe.

To confirm the appearance of multiple D-gene segments in the genome we digested total human placental DNA with BamHI and probed it with the 1.8-kb germ-line D fragment. As seen in Fig. 2a, this probe detects not only itself, but also four additional BamHI fragments of about 1.6, 6.6, 7.4 and 18 kb length. We conclude that D genes are indeed multiply encoded within the genome and are organized into gene families, reminiscent of V-gene gamilies.

# D genes are tandemly arranged on the genome

To learn more about the organization of D-gene segments we have mapped our phage clones with the restriction enzymes BamHI, HindIII and EcoRI. Two phage isolates are indistinguishable from each other (A7 and A4) and five different phage inserts overlap each other. These overlapping phages link four different D genes to each other on a 33-kb long DNA segment, as shown in Fig. 3. One additional phage, A6, is as yet unlinked and contains one D segment.

The map of this D-gene family locus reveals a surprisingly regular tandem arrangement of D genes, separated from one another by about 9 kb. It is not known whether these large spacer regions are functionally significant. Three D-containing genomic fragments of 1.6, 1.8 and 6.6 kb, detected in a BamHI digest of total placental DNA, are also present in our phage inserts. The remaining two genomic fragments of about 7.4 and 18 kb are likely to carry D3 and D5, located on the phages A3 and A6, respectively. In both cases, the clone inserts end within the genomic BamHI fragments. Consequently we have cloned all five identifiable members of this D-gene family, four of which are regularly spaced ~9 kb apart.

We established the 5' to 3' order of these gene segments by analysing genomic DNA from chronic lymphocytic leukaemia cells (CLL-LR). As discussed above, these differential leukaemic B cells have rearranged both chromosomes to give rise to an active (LR-35) and to an aberrant (LR-36) recombinant. Assuming that recombination is accompanied by a deletion of intervening DNA<sup>22</sup> and that D genes reside between V and J genes, the chromosome carrying the active V-D-J gene should not contribute any germ-line D-gene fragments. The chromosome carrying the aberrant D-J6 recombinant, however, is expected to have retained those germ-line D-gene fragments located 5' to the aberrantly recombined D, while deleting those fragments located 3'.

Accordingly, we probed a BamHI digest of DNA from CLL-LR with the 1.8-kb BamHI fragment which contains germ-line D sequences; a DNA digest of a fibroblast line established from the same patient served as a germ-line control. As Fig. 2c shows, the D-bearing germ-line fragments of 1.6, 1.8 and 7.4 kb length have been deleted in the leukaemic B cells relative to the germ-line pattern (Fig. 2b). This orders the retained 6.6-kb long, D4-bearing fragment to the 5' side of the deleted 1.6-kb (D1), 1.8-kb (D2) and 7.4-kb fragments (see Fig. 3). According to the map order in Fig. 3, D3 must be deleted along with D1 and D2 and must therefore reside on the 7.4-kb germ-line fragment. (The only other genomic band, 18 kb long and as yet unlinked, is not deleted in this B cell.)

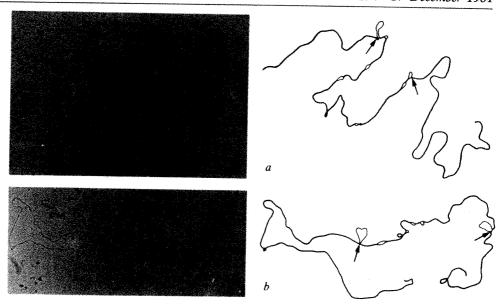
# D regions are embedded in 9-kb repeat units of DNA

To identify possible homologies around D-gene segments, we heteroduplexed phages A5 and A3 with each other. Inserts within A5 and A3 do not overlap and so any heteroduplex formed between them is indicative of sequence homology, but not identity. Surprisingly, the two phages form a heteroduplex structure not only around the D segments, but almost throughout their entire lengths (see Fig. 4a). This extensive homology is interrupted only by several small nonhomologies in addition to deletion loops at the ends of inserts; such deletion loops are expected because different clones are unlikely to end in similar positions within the homology. Several of these small regions of nonhomology vary in size between molecules and occasionally are not visible, suggesting that even these regions have some homology, although not usually sufficient for heteroduplex formation.

Similarly, we can demonstrate homology between all the spacer regions surrounding these D segments. For example, the overlapping phage inserts of A3 and A2 (see Fig. 3) heteroduplex in a way which shows the spacer homology rather than identity of sequence in the overlap; such a heteroduplex is shown in Fig. 4b.

This locus of the human genome then appears to consist of 9-kb long repeat units, apparently arranged end to end, with each unit encoding one D-gene segment of this family. Such an organization is likely to be the result of very recent duplication

Fig. 4 Electron micrographs and schematic representations heteroduplexes formed betweeen phage clones containing D segments. Methods for heteroduplex formation have been described elsewhere Phages A5 and A3 form an extensive heteroduplex, although their inserts do not overlap (see Fig. 3). Spacer DNAs between these D segments are homologous to each other. b, Phages A2 and A3 similarly form an extensive duplex. Pairing of the imperfect, though large spacer homology is preferred over pairing of the smaller region shared by these two overlapping phages (see Fig. 3). The spacer homology for both a and b is interrupted by a few small mismatched regions. The extent to which some of these small mismatch loops form varies slightly between heteroduplex molecules, although the overall pattern of these mismatch



regions is preserved and is found reproducibly in more than 10 molecules examined. Phage insert end are indicated by arrows. Deletion loops at the insert ends merely show that the two phage clones do not end at identical positions within the homology.

events. Nonetheless, mechanisms that operate to maintain sequence homologies within the spacer cannot be ruled out.

#### Germ-line D-gene segments are surrounded by recombination signals with 12-bp spacers

To establish formally the existence of D segments on our clones and to allow further characterization, we sequenced the relevant regions using the Maxam–Gilbert procedures. Figure 5 shows four such embryonic D sequences of this family. All are surrounded by recombination signals with a 12-bp spacing between the heptamer and nanomer sequences. This allows for recombination with signals of about 23 bp spacing, as are found on the 3' side of  $V_{\rm H}$  genes<sup>7,8,23–25</sup> and the 5' side of  $J_{\rm H}$  segments<sup>7,8,20,26</sup>, and as would be required by the 12/23-bp spacing rule.

The sequences within and surrounding these D segments are closely homologous to each other, differing mostly by point mutations, except for a deletion of a triplet in D3. Most of the nucleotide differences lie within the potential coding sequences (that is, between the recombination signals) and in particular near the middle of these segments. The recombination signals and spacers, on the other hand, particularly those on the 5' side, are very strongly conserved. Surprisingly, the 5' and 3' spacer sequences are quite complementary to each other and, together with the complementary recombination signal, an extensive stem structure could be drawn, looping out the D-coding segment.

D sequences of different families are heterogeneous in length. The presumptive coding blocks of this family of D segments are 31 bp long (D3 has 28 bp). DHQ52, a D segment interspersed

among J regions in man and 5' to J segments in mouse, is 11 and 10 bp long, respectively 18,20; DSP2.2 in mouse encompasses 17 bp 19. Observed size variations in the third hypervariable region of heavy chains 27 may reflect length heterogeneity of D segments.

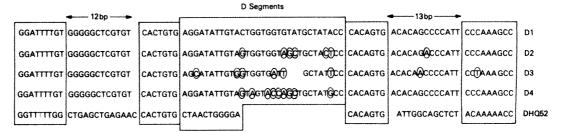
The diversification of variable regions contributed to by D segments increases even further when we consider the inherent flexibility associated with recombination events<sup>14,15</sup>. Flexibility in the exact point of joining has been demonstrated for V-J recombination in light chains; for example, V and J segments are sometimes joined out of phase with each other<sup>28</sup>.

In contrast, D segments have no known coding frame. Therefore, if we assume a flexible recombination mechanism, all three reading frames of D sequences are potentially usable. This would further diversify immunoglobulins of different B cells. In this context, all three reading frames of D1 are open, that is, they do not contain any stop codons, while D2, D3 and D4 each have two open reading frames.

# Is aberrant D-J the result of more than one recombination event?

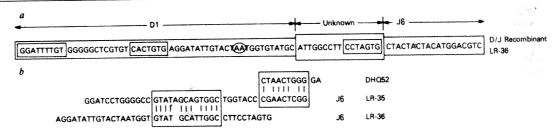
As discussed above, only D1 can be the parent of the aberrant D-J6 recombinant. Germ-line DNA between D1 and J6 has been deleted during recombination (Fig. 2c) and sequence analysis confirms this assignment (see Fig. 6a). Sequences within 200 bp 5' of D1 are identical to those 5' to the D-J6 recombinant, as determined by restriction mapping and partial sequencing. All other members of this family (including D5 on phage A6) exhibit similar, but clearly different sequences.

Fig. 5 Nucleotide sequences of four members of this human D family (D1, D2, D3, D4) in addition to DHQ52, published elsewhere<sup>20</sup>. The D4, D2 and D3 sequences are compared with D1 and differences are shown by gaps or encricled bases. The



the D segments are boxed—all contain a 12- or 13-bp spacer between the heptamer and nanomer parts. No other recombination signals are found in the immediate flanking sequences (not shown here). All four D segments were sequenced according to the Maxam and Gilbert procedures<sup>35</sup>. D1 was sequenced from a 400-bp long BamHI-HindIII fragment, whereas D4, D2 and D3 were sequenced from 800-bp long BamHI-HindIII fragments (see Fig. 3). (These fragments were obtained by restriction of subcloned BamHI fragments (see Fig. 3).) D segments were located by random sequencing within these fragments. Sequences were determined in both directions. The four D segments are much more closely homologous on their 5' sides than on their 3' sides, although they all show extensive homology except near the middle of the coding segments. D3 has deleted a triplet whereas all other differences are point mutations.

Fig. 6 a, Parents of the D-J recomaberrant LR-36. The binant nucleotide sequence of I R-36 is derived entirely from the D1 parent and J6 parent except for 16 bp immediately adjacent to J6 (designated 'unknown' here) and 2-bp mutations 10 bp 5' to the



unknown DNA (circled here). This novel DNA contains a heptamer-like recombination signal and may represent the 3' end of an unidentified D segment which recombined with D1. Recombination signals are boxed. b, The active recombinant LR-35 sequence shows segmental homologies with the LR-36 sequence and the DHQ52 germ-line segment. Homology regions are boxed. LR-35 seems to derive parts of its sequence from D segments also used by the aberrant recombinant LR-36, but in addition shows homology to DHQ52. This suggests that different D segments may have recombined with each other to form a mosaic LR-35 sequence. (Interestingly, the region shared by LR-35 and LR-36 spans the presumed recombination point in LR-36 (see part a of this figure).)

Although most of the coding segment of D1 is preserved during recombination, the six 3'-most base pairs of D1, are not found in the recombinant. Instead, 16 bp of unknown origin appear directly adjoining J6 (see Fig. 6a). What gave rise to these sequences? As this sequence arrangement is not present in germ-line DNA, we must invoke an additional rearrangement: D segments may be able to recombine with each other. Within the 16 bp of unknown origin we find a heptamer-CCTAGTG—which resembles the heptamer consensus sequence for recombination signals. This may be a remnant of the 3'-recombination signal attached to an unknown D sequence which recombined with D1; joining to J6 did not completely eliminate the signal.

The parent sequence D1 and the recombinant LR-36 also differ in two adjacent nucleotides within the otherwise preserved D sequence (10 bp 5' to the presumed D-D joining site, see Fig. 6a). These point mutations might have arisen somatically, either connected with or independent of the recombination event. Also possible is a polymorphism in the DNA sequence of this patient. Although less likely, we cannot rule out the possibility that D1 recombined with another D 5' to the two nonmatching nucleotides. This would require part of the unknown D segment to be identical with D1.

The presumed active recombinant LR-35 provides further, albeit indirect, support for the D-D recombination proposal. Part of the LR-35 gene sequence is quite homologous to a sequence in the D segment of the aberrant recombinant LR-36; beyond this homology region, the two sequences diverge. However, the LR-35 D segment also has some additional homology with another germ-line gene segment, DHQ52. This homology is 3' to the homology it has with LR-36 (see Fig. 6b), which suggests, but does not prove, that at least three different D segments have contributed to the LR-35 D; that is, that multiple recombinations between D segments can generate active Dregion segments.

At present we do know how D segments recombine with each other because no appropriately situated recombination signals exist to accomplish such joining. In light of existing recombination events, we would expect recombination signals conforming to the 12/23-bp spacing rule next to the eventual joining sites of the two D segments. We see no such signal next to the presumed site of rearrangement in the germ-line D1 sequence. On the other hand, it has been suggested that such a signal exists in some mouse segments29. It is possible, however, that the extensive homology that surrounds the D-region sequences has a role in their interaction, either somatically or within the germ line. Such a mechanism, involving homologous recombination or gene conversion, would allow segments to amplify their germ-line diversity in much the same way as has been proposed for members of V-region families<sup>12</sup>. Clearly, the resolution of this question will depend on the further analysis of germ-line and rearranged D-region genes.

#### D-gene segments can generate enormous diversity

The variable region of the heavy chain derives its diversity from four principal sources: multiply encoded V-, D- and J-gene segments; a combinatorial joining mechanism; flexibility in the exact site of recombination that can generate different amino acids at junctions between joined segments (in the case of D segments, this flexibility allows reading in different frames); and a potential for solitary somatic mutations, an additional process now being documented<sup>24,25,30,31</sup>

The most diverse portion of the heavy chain is the third hypervariable region. This is understandable in terms of what we know of D-region genes. Not only are D segments multiply encoded, but these short sequences of variable length might also recombine with each other. Flexibilty at the site of joining further diversifies junctions between V, J and other D regions and could create different reading frames within D segments. This system is therefore able to generate an enormous amount of coding information, particularly within the third hypervariable region, providing a major source of diversity.

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#### HR 465 returning to rare-earth-maximum phase

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HR465 is unusual among Ap stars. Its spectroscopic variability has been discussed by Wolff and Preston<sup>1</sup> who show that at the rare-earth-minimum phase, the sharp-lined spectrum is similar to that of many other Ap stars of the 'magnetic sequence'. The rare-earth-maximum phase, studied Bidelman and Pyper (personal communication), was found to have an extremely high density of lanthanide rare-earth lines in addition to Hg II 3,984Å. Their observations of this phase were made in the early 1960s and because of the very long period of the star, around 22-24 yr, they have not been repeated. Until recently, the critical phase of HR465 has been unavailable. However, we report three 2.4Å mm<sup>-1</sup> spectrograms of HR465 taken at the Dominion Astrophysical Observatory (DAO) in August 1981 which show that the spectrum now very closely resembles that of the early 1960s—the rare-earth-maximum phase has returned.

Aller and Cowley's<sup>2</sup> announcement of the possible presence of the unstable element promethium, whose longest-lived isotope has a half life of only 18 yr, in the spectrum of HR465 caused much interest. Setting aside this controversy<sup>3,4</sup>, the rareearth-maximum spectrum of HR465 is of great interest. The elemental abundance patterns<sup>5,6</sup> suggest the presence of r-process peaks. Ohnishi<sup>7</sup>, and Steinberg and Wilkins<sup>8</sup> have discussed possible interpretations of these abundances in terms of fission. There are strong arguments<sup>9</sup> for the presence of the actinide rare earths uranium and thorium. An unusual distribution of wavelength residuals suggest that the uranium may contain a high percentage of isotopes lighter than 238. A possible uranium-to-thorium ratio greater than unity could be interpreted in terms of a recent r-process event, and the excess uranium would then be due to the presence of isotopes lighter than 238 (ref. 10).

The star has been monitored at the DAO since 1974. In 1979 and 1980, substantial increases in the line density were noted, as well as a progressive strengthening of lines of the heavier lanthanides, notably dysprosium. Particular attention was paid to the feature near 3,859.6Å, which closely coincides with the wavelength of the strongest U II line11. This feature, which is dominated by Cr I in most magnetic Ap stars, weakened througout the late 1970s along with the Cr I and II spectra. There is a pronounced weakening of Cr I throughout this period.

In the August 1981 spectra, 3,859.6Å has strengthened considerably; it may now be considered a moderately strong line (~80 mA). Figure 1 shows tracings of the region of UII 3,859.6Å made from spectrograms taken at the previous rareearth maximum (A), an intermediate epoch (B), and in August 1981. The top two tracings have been described and detailed line identifications given elsewhere<sup>12</sup>. Figure 1C shows DAO plate no. 13891, taken in August 1981. Filled squares below the absorption lines mark features which have been wholly or partially identified as due to lanthanides. While most of the features in the rare-earth-maximum spectrum are marked with squares, only three features are so marked in Fig. 1B, where many of the absorptions are due to chromium.

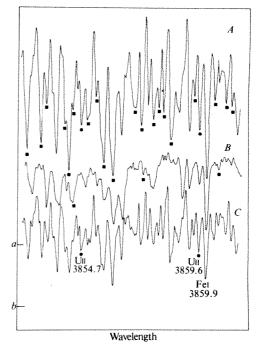


Fig. 1 Direct intensity tracings of HR465 taken on September 1961 (A), November 1974 (B) and August 1981 (C), in the region of the strong U II lines at 3,854.7 and 3,859.6Å (•). The strong Fe I line is at 3,859.91Å. Lanthanide lines are indicated by solid squares (). A one-to-one correspondence of features in the A and C is apparent. Zero intensities for A and B are marked a and brespectively. The base is zero for C

Wavelengths for one of the current DAO plates have been measured from PDS scans using a program developed by Rice. Positions for 4,143 lines in the region 3,707-4,655 Å were analysed by the method of wavelength coincidence statistics (WCS)<sup>13</sup>. Essentially the same heavy ion species that were present at the last rare-earth maximum can again be identified. In particular, dysprosium (Dy III is especially strong), Ho and Er are identified at very high confidence levels. The 1981 plate shows good statistical evidence for the presence of osmium, and marginal evidence (98% confidence) for platinum. The presence of both of these transition metals has been found at less exotic

WCS of the 1981 plates show marginally significant results for U II (97.5% confidence), Th II (97.5% confidence) and Th III (99.5% confidence).

The present observational material (2.4Å mm<sup>-1</sup>) is not good enough to allow us to extract definitive information on the critical isotopic abundance ratios. Very high resolution and low noise are needed. Many features must be studied because of the severe problem of blends.

For the next few years, HR465 will be in a critical phase which deserves intensive investigation.

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## Supernovae and nitrate in the Greenland Ice Sheet

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Nitrate concentration in the absolutely dated Greenland ice core from Crête has been measured for six time intervals, five surrounding the time of appearance of the well established historical supernovae during the past 1,000 yr and one during the Maunder minimum of solar activity, to look for a possible correlation between supernovae and nitrate concentration. The findings of Rood et al. of nitrate spikes corresponding to the appearance of the historical supernovae and a pronounced minimum in nitrate contents during the Maunder minimum are not confirmed. A very regular annual variation of nitrate concentration is observed superimposed on a constant background. We show here that both these signals seem unaffected by the known variations in the solar activity for the periods analysed. The nitrate contents are unaffected by peaks in acidity caused by volcanic eruptions. The annual variation of nitrate concentration suggests it could be used for dating ice cores.

Nitrate deposited in the Antarctic Ice Sheet has attracted attention because of its possible production by the absorption of ionizing radiation from extraterrestrial sources in the stratosphere 1-3. The deposition rate of nitrate in snow accumulated in areas remote from soil and sea should then reflect the influx of radiation from space, modulated by atmospheric processes such as exchange between the troposphere and stratosphere and the general tropospheric circulation. If this were true, the concentration of nitrate in polar glacial ice may contain information on solar activity variations, galactic cosmic-ray influx and changes in the atmospheric circulation connected with climatic changes.

Rood et al. have discussed whether the flash of X rays from a supernova explosion in our Galaxy might produce sufficient NO in the stratosphere to produce a spike of nitrate in ice cores a year or two after the event. In an Antarctic ice core they report four spikes with a factor of 2-3 higher than the background level. Three of the spikes seem to coincide reasonably well with the supernovae observed in 1181, 1572 and 1604. We know of no absolute dating for the South Pole station core in question and the expected accuracy of the dating is not reported by Rood et al.1. Three proposed datings differ ~40 yr for the AD 1181 event. The fourth nitrate event, at about 1300, is not correlated with any known historical supernova. Rood et al. report further indications of variation of the background level with the solar cycle and claim a pronounced low level of nitrate coinciding with the Maunder minimum of solar activity.

The perspective of these observations and the availability of the absolutely dated Crête ice core, induced us to measure nitrate in the Greenland ice. The fact that the historical supernovae predominantly lighted the Northern Hemisphere suggests that detection is more likely in Greenland than in Antarctica.

Supernova explosions are rarely observed in our Galaxy: Table 1 lists the data for the five historical supernovae observed in the past 1,000 yr. Data are partly historical and partly from recent optical and radio observations of the remnants4.

A supernova, Cas A, is believed to have occurred around 1750, but as the time of appearance is based only on extrapolation of present-day observations of the remnant it is not considered here. The next candidate before 1006 is AD 393 (ref. 4) which is out of range of the ice core material available for this study. However, the material does include the Crab supernova of 1054 and notably the year 1006 event, which is the one closest to the Solar System and the brightest new star ever observed.

The 404-m polar ice core used in this study was recovered using a thermal drill in a dry hole in Central Greenland 71 °N, 37 W in 1974 by the US Cold Region Research and Engineering Laboratory under the US-Swiss-Danish program Greenland Ice Sheet Program (GISP). The core handling and storage procedures have been described by Langway<sup>5</sup>, and the sample preparation by Hammer<sup>6</sup>. For the time intervals in question the ice core is dated to an accuracy of ±1 yr back to AD 1104 and  $\pm 3$  yr back to AD 553, which is the age at the bottom of the core. The dating is done by counting seasonal variations of the stable oxygen isotope ratio,  $\delta^{18}$ O, and acidity downwards from the surface'. The acidity curve provides the time scale with fix points back to AD 1104 using historical accounts of volcanic activity in the Northern Hemisphere. A volcanic eruption causes an increase of the acidity of the polar ice, the size of the signal found in the ice depends on the magnitude and geographical latitude of the eruption. All sample collections are based on continuous sequences of 12 ice samples per calculated annual layer for  $\delta^{18}$ O and acidity, and in general of four samples per calculated year for nitrate. The theoretical thickness of an annual layer at a given depth is calculated from a flow model for the ice sheet7. The mean annual accumulation at Crête is 29 cm of ice and the thickness of a calculated year AD 1000 is ~25 cm. Extreme care was taken to ensure no contamination of the samples for nitrate analysis. Samples of about 10 ml were cut in a laminar flow bench and a microtome knife used to clean the ice surfaces. Several centimetres of ice were cut away from the surface of the core. The samples were taken from crack-free regions of the core. The clean samples were put into sealed plastic beakers and kept at -20 °C. Melting was done just before the chemical analysis for nitrate.

The nitrate concentration (given as  $\mu$ equiv. NO<sub>3</sub> per kg ice) was measured as nitrite after reduction in a cadmium column. The nitrite was converted into a form of an azo dye by adding sulphanilamide and n-(1-naphthyl)-ethylene diamine dihydrochloride. The optical absorption of the dye solution was measured. The reproducibility of the method,  $\pm 0.02$   $\mu$ equiv.  $kg^{-1}$  (±2%), was achieved during the single runs of analysis. For comparison of the absolute levels between the six series of meaurements an accuracy of 5% was obtained: the source of error was the uncertainty in the dilution factor for the standard solutions used for the calibration. The measured quantity is the sum of nitrate and nitrite. The concentration of nitrite was measured on a few of the samples by bypassing the cadmium reduction column. The contents were always consistent with zero within the quoted measuring accuracy. The measurements are therefore assumed to give the nitrate concentrations.

Figure 1 shows the results of the nitrate analysis for the six short series of the selected time intervals covering the years before and after the appearance of the historical supernovae listed in Table 1. Also shown are curves giving the acidity from conductivity measurements together with  $\delta^{18}$ O data. The  $\delta^{18}$ O

Table 1 Parameters of five historical supernovae Posttion (1950) Distance Vanbility Name RA Dec (kpc) 15 10 <del>-4</del>0 1.3 I

1006 3 April 2 years 1054 4 July Crab 05 40 +20 22 months 1181 6 August 1572 8 November 01 30 +658 185 days 00 20 Tycho +65 I 16 months 1604 8 October 17 30 -20-3 10 Kepler 12 months

Date

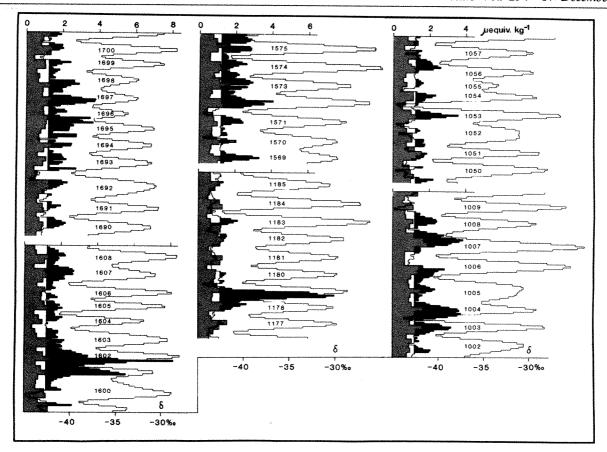


Fig. 1 Three curves for each of the six time intervals. Nitrate concentration (stippled area: left-hand scale  $\mu$ equiv. NO $_3^-$  per kg ice). Acidity fallout (black area: left-hand scale  $\mu$ equiv. H $^+$  per kg ice). Acidity above the background 1,2  $\mu$ equiv. kg $^{-1}$  are due to fallout of volcanic acids, mainly H<sub>2</sub>SO<sub>4</sub> and HCl, from eruptions in the Northern Hemisphere.  $\delta^{18}$ O to the right with scale at the bottom (per  $10^3$ ).

curve is first order corrected for diffusion  $^{10}$ . The beginning of a calendar year is arbitrarily defined as the point where the corrected  $\delta^{18}$ O begins to rise. The nitrate curve is represented by a mean of four samples per calendar year. By distributing and assigning the measured amount of nitrate to the calendar years defined above, one arrives at the mean nitrate concentration in calendar years. Figure 2 shows the annual deposition determined in this way. At the end of each curve the mean value and the standard deviation of the annual values are indicated. The bars reflect the variability of the phenomenon, the experimental errors in the chemical analysis being an order of magnitude smaller. The uncertainty in placing the divisions between calendar years also contributes to the standard deviation. No significant enhancement beyond the normal variations in nitrate

deposition is observed in the years of the five supernovae or the years following these events. From the accuracy of the absolute dating we conclude that the analysed portions of the ice core cover the times of appearance of the five supernovae. The curve for the interval AD 1690-1700, which is selected to represent the Maunder minimum, shows that nothing unusual is taking place, the mean deposition of nitrate is at the same level.

The correlation between nitrate and  $\delta^{18}$ O data is good. Like  $\delta^{18}$ O the nitrate shows a regular annual variation that is clearly visible, although there are only four samples per calculated year. Both curves indicate concordantly the fluctuations in the annual amount of precipitation. The annual variation in nitrate deposition seems to be the same for the six time intervals observed, sampling a period of 700 yr. Both the variation and the mean

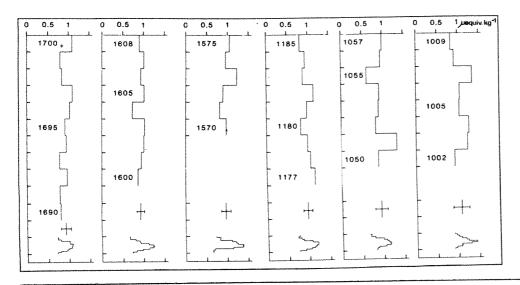


Fig. 2 Calendar annual means of nitrate concentration in μequiv. kg<sup>-1</sup>. The bars indicate the formal standard deviation and mean value of the calendar values, that is they do not reflect the experimental precision of the chemical analysis. The inset at the bottom shows the mean annual variation calculated by signal averaging from the original data of Fig. 1.

level in nitrate deposition seem to be independent of the recognized variations in solar activity during the Maunder minimum<sup>11</sup>. Figure 2 also shows the mean annual variation curves in nitrate deposition which were obtained by a signal averaging procedure<sup>12</sup>. From the measurement in Fig. 1, 12-monthly values are read evenly distributed in each calendar year. Average values are then taken for each month. The curves indicate an increase in nitrate deposition in the spring and a decrease in the autumn. The trough to peak variation is ~0.5  $\mu$ equiv. kg<sup>-1</sup> and the background is ~0.75  $\mu$ equiv. kg<sup>-1</sup>.

There seems to be no variation of the amplitude of the annual oscillation with an 11-yr sunspot cycle. The solar activity is, however, not well recorded in pre-telescopic time11 and conclusions must wait for a comparison with nitrate data from a

longer and more recent period.

The six time intervals contain two events in acidity ascribed to volcanic eruptions9. One is in AD 1178-1179 and correlated with an eruption of Katla, Iceland. The other is in AD 1601-1602 and not identified. Note that the nitrate curve goes undisturbed through the volcanic peaks. The acidity peaks are mainly composed of sulphuric and hydrochloric acids

The regular annual oscillation in nitrate contents makes it in principle possible to date ice cores by observing the nitrate signal, especially in polar ice from regions where no summer melting occurs (such as at Crête) and thus no percolating melt water penetrates the snow and disturb the stratified layers. We suggest that the higher deposition rate of nitrate in the summer is due to a stratospheric fallout following the opening of an tropospheric-stratospheric exchange every spring. The modern nitrate deposition in rain water in areas of Scandinavia, remote from industrialized regions, is at the level of 2  $\mu$ equiv. kg<sup>-1</sup>, and the natural pre-industrial deposition rate can be set to the level of 1 µequiv. kg<sup>-1</sup> judged from the lowest observations in northern Scandinavia<sup>13</sup>. The winter level of deposition in the Greenland ice is of the same magnitude and is therefore consistent with a residual tropospheric contribution in the clean air conditions above the Greenland ice.

It has been suggested that supernovae occurring close to the Solar System will enhance production of NO in the stratosphere and consequently produce observable nitrate peaks in ice cores<sup>14,15</sup>. As shown here the supernova (SN1006) occurring as close as 1,300 pc, caused no such effects in the Greenland ice. Candidates closer than that are the supernovae corresponding to the young short period Vela pulsar, the Lupus Loop, the Cygnus Loop and the North Polar Spur. The ages of these supernova remnants are estimated to be in the range 10,000-100,000 yr 16 This matches the time interval for which glacial material is available from the Antarctic and Greenland Ice Sheets. A local bubble of hot interstellar material observed in soft X-rays is considered to have originated from a supernova having exploded very near (<18 pc) the Solar System (R. Rothenflug, M. Arnaud & A. Soutoul, personal communication and ref. 17). If nitrate events in ice cores could be found and correlated with close supernovae an accurate dating of these is possible using the glacial record, and information on emission of  $\gamma$  rays and cosmic rays and their propagation to the Solar System could be obtained.

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## Deuterium/hydrogen ratios in unequilibrated ordinary chondrites

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The discovery of oxygen of anomalous isotopic composition in the Allende meteorite during the early 1970s led to the detailed investigation of primitive materials for unusual isotopic signatures. One element studied was hydrogen; major enrichments in deuterium relative to terrestrial and proposed nebula compositions have been discerned by close examination of specific phases within various meteorite samples. Here we report high and variable deuterium enrichments in two of the most unequil-Ibrated (unmetamorphosed) type LL3 ordinary chondrites, Bishunpur and Semarkona. Water released from bulk unseparated material by pyrolysis has  $\delta D$  values up to  $\sim 3,000\%$ (SMOW) or in absolute terms a D/H ratio of  $\sim 6 \times 10^{-4}$ . Thus, one of the highest deuterium contents yet encountered for a Solar System species has been observed without considering a specific fraction of a sample. Either these meteorites contain a greater proportion of the D-rich phase first recognized in meteorite separates, or a small fraction having an even more extreme deuterium content resides within them.

Variations in deuterium abundance are usually small and are reported relative to standard mean ocean water (SMOW):

$$\delta D = \left[ \frac{(D/H)_{\text{semple}} - (D/H)_{\text{SMOW}}}{(D/H)_{\text{SMOW}}} \right] \times 1,000\%$$

Hydrogen in terrestrial waters and other materials has a range of isotopic compositions falling within several hundred per mil of the absolute D/H ratio  $(1.56 \times 10^{-4})^2$  of SMOW<sup>2</sup>.

Before 1977 only two studies of meteorite D/H ratios had been attempted. Both concerned carbonaceous chondrites and had given inauspicious results in terms of anomalous compositions. Boato<sup>3</sup> recognized adsorbed or terrestrially exchanged H<sub>2</sub>O which could be removed by heating at temperatures up to 200 °C and more significantly, he found hydrogen (probably from hydrated silicates and organic matter) released at higher temperatures was enriched (up to 300%) in D relative to SMOW. Briggs<sup>4</sup> studied D/H ratios of solvent extractable organic materials; again enrichments up to 200% were encountered which led him to argue for an extraterrestrial origin for the compounds investigated.

More recently Robert et al.5 have studied ordinary chondrites in an attempt to differentiate between water from hydrated minerals and from the decomposition of organics. All the samples considered, with the exception of Chainpur, an LL3 chondrite, were within the terrestrial range. Subsequent investigations  $^{6,7}$  with material separated from Chainpur suggest the existence of a phase(s), labile between 450 and 750 °C and occurring with or within chondrules, which has a  $\delta D$  value of +2,000 to +4,400%; that is, containing a factor of ~5 times more deuterium than SMOW, an enormous enrichment by terrestrial standards. Kolodny et al.<sup>8</sup> reappraised the organic hydrogen in carbonaceous chondrites and postulated the presence of organic material in the meteorite Renazzo with a &D of +1,600% relative to SMOW. This effort was criticized 9.10 on

the grounds that the low-temperature oxygen plasma used to remove the organics selectively, in fact removed more hydrogen than could be accounted for by the original organic content; the material balance calculations used by Kolodny et al.<sup>8</sup> did not allow for this. Robert et al.<sup>9</sup> and Robert and Epstein<sup>11</sup> investigated extractable and polymeric organics from the Murray meteorite and measured directly D enrichments approaching +1,000% in this specimen, whereas Smith and Rigby<sup>10</sup> obtained  $\delta D$  values of +450% for acid insoluble residues from several carbonaceous chondrites.

While carbonaceous chondrites have been extensively studied for their hydrogen isotopic contents, primitive unequilibrated ordinary chondrites, except for Chainpur, have largely been ignored. It is now accepted 12.13 that class 3 unequilibrated meteorites may be further subdivided according to the degree of unequilibration 14; the number of subdivisions may be up to 10 (ref. 12). Although there is some disagreement as to whether Chainpur is one of the most unequilibrated examples of the class, it is accepted that three LL3 samples, Bishunpur, Krymka and Semarkona may be considered the most primitive 12.13. If the anomalously heavy hydrogen in Chainpur is a pre-Solar System remnant, it might also be found in these particular specimens.

Preliminary experiments on material from close to the fusion crust of a small sample from Bishunpur showed that water could be extracted with a  $\delta D$  value of between +2,489 and +3,109% SMOW (ref. 15). These results are for water released above 200 °C (to avoid terrestrial contamination<sup>3,7</sup>) and below 750 °C, the temperature by which most water has been released from Chainpur<sup>7</sup>. Comparable sized samples of local meteoric water transferred through the same apparatus and distilled water used for cooling when cutting the meteorite gave  $\delta D$  values typical of the latitude of the laboratory (-44 and -48% SMOW respectively).

The latter experiments are not a true procedural check because temperatures up to 750 °C were not used. Because the Cambridge laboratory uses deuterium for various labelling purposes, we aimed to demonstrate the validity of the preliminary results. Our experimental procedure is: for each water extraction we used a new quartz reaction vessel prebaked to >1,000 °C overnight on a line run at 200 °C. During water collection this vessel is valved off from the pumping system. Before sealing the extracted waters in a quartz takeoff finger (trapped by liquid N<sub>2</sub> for 2 h) for transfer to East Kilbride for isotopic measurements, non-condensable gases are pumped away by opening the valve to the high vacuum line for  $\sim 10$  s. There is no possibility of back trapping of water from the vacuum system during this pump-out because of the presence of a protecting liquid N<sub>2</sub> trap immediately next to the valve on the system side. During our experiments aliquots of a terrestrial biotite were processed alternately with the samples not as a measure of reproducibility, but as a contamination check.

All water samples were converted to hydrogen using the conventional hot uranium technique<sup>16</sup>. Isotopic analysis of hydrogen was performed on a VG Micromass 602 mass spectrometer using a reference of  $\delta D = -50\%$  relative to SMOW, produced from doubly distilled water and calibrated against the international standards V-SMOW and V-SLAP. The memory correction associated with reducing waters widely separated in  $\delta D$  was estimated from V-SMOW and V-SLAP analyses to be 1.5% and this has been applied to all meteorite data.

All our D/H data from unequilibrated ordinary chondrites are listed in Table 1. Bishunpur and Semarkona release water extremely enriched in deuterium. The biotite blanks run alternately with the samples indicate the results obtained are neither an analytical artefact nor the manifestation of deuterium contamination introduced in the Cambridge laboratory. The spread in biotite  $\delta D$  values is considerably greater than that of replicate total hydrogen extraction ( $\pm 2\%$ ,  $1\sigma$ ) and is attributable to incomplete yields for biotite using the experimental technique devised for meteorites.

Sample inhomogeneity is common on all scales in the least equilibrated ordinary chondrites<sup>14</sup> and may explain the dis-

Table 1 D/H data from unequilibrated ordinary chondrites

	Wt		$\delta D\%$
Sample	(mg)	wt % H <sub>2</sub> O <sup>8</sup>	(SMOW)
Bishunpur 1*	~200	ND	+2.489
Bishunpur 2*	81.9	ND	+3,109
Biotite	21.0	ND	-35.3
Krymka†	98.7	ND	+54
Biotite	20.5	ND	-67.8
Semarkona‡	31.1	0.34	+2,904
Biotite	31.7	0.74	-62.6
Bishunpur 3†	31.2	0.29	+1,101
Biotite	30.4	0.71	-77.3 <sup>"</sup>
Biotite	36.4	0.79	-21.0
Weston†	37.7	0.23	+16.1
Biotite	30.7	0.60	-13.8

<sup>\*</sup> Supplied by Dr S. O. Agrell (University of Cambridge) run with alternating tap water samples 15.

crepancy between the Bishunpur 3 result and those of the preliminary study<sup>15</sup>; Bishunpur 3 was obtained from an alternative source to the other two aliquots and the amount analysed was substantially less. The apparent inhomogeneity of Bishunpur may be significant and might indicate a sporadic occurrence of a minor very D enriched phase. We cannot explain the Krymka result which seems to suggest that the occurrence of anomalously heavy hydrogen is not simply a question of degree of unequilibration, although very inhomogeneous distribution of D rich phases cannot be ruled out. Water extracted from dark portions of the nominally H4 chondrite Weston, which has H3 type inclusions<sup>17</sup> and water present in veins of iddingsite<sup>18</sup>, does not exhibit an extreme D content. However, Weston has a compliment of solar wind gases<sup>19</sup> and any hydrogen present from the solar wind might be expected<sup>20,21</sup> to have a D/H of  $<3\times10^{-6}$  possibly counterbalancing a component enriched in D. The  $\delta D$  value obtained for Weston in this study (+16%) is greater than that of -100% reported by Robert et al.<sup>7</sup>

Our results are entirely for bulk unseparated meteorites. No attempt has been made to preconcentrate or selectively decompose the host phase of the water analysed. Alternative explanations for the Bishunpur and Semarkona data are either: (1) D enrichment is ubiquitous and all the hydrogen turning up in the water extracted has an isotope composition approaching that observed in certain Chainpur separates or (2) somewhere in these very unequilibrated chondrites is a phase of exceptionally high D content. In the case of Chainpur, stepwise heating and selection resulted in a  $\delta D$  increase from +200% for bulk material<sup>5</sup> to +4.400% for the 450-750 °C temperature cut from several chondrules<sup>7</sup>. If explanation (2) is correct for Bishunpur and Semarkona, a comparably successful future isolation study ought to reveal a truly extreme hydrogen isotopic composition. Either way, studies of the material separated from Bishunpur and Semarkona should help to identify the D-enriched host phases. A possible site for a D-rich phase surviving accretionary processes might be the opaque matrix reported by Huss et al.1 and thought to be the low temperature condensate of Larimer and Anders22

The problem of D-enriched molecules in primitive meteorites has been extensively discussed elsewhere  $^{23}$ . It might have been easier to accept deuterium depletions as the protosolar nebula is thought  $^{24}$  to have had a D/H ratio of  $2.5 \times 10^{-5}$  and unmetamorphosed meteorites could have been expected to have retained some original material. Geiss and Reeves  $^{23}$  concluded ion-molecule reactions at low temperatures to be the most likely means of concentrating deuterium, and inferred that the deuterated species in Chainpur and carbonaceous chondrites were remnants of dark interstellar clouds as first suggested as a possibility by Kolodny et al.  $^8$ . We must concur with this interpretation

<sup>†</sup> Supplied by Dr R. Hutchison (British Museum).

<sup>‡</sup> Supplied by Dr R. Clarke (Smithsonian Institution).

<sup>§</sup> Measured manometrically as H2.

<sup>||</sup> Corrected for memory contribution of 1.5%, from hydrogen  $\delta D \sim -40\%$ . ND, not determined.

as it is very unlikely that an elementary experiment such as ours has recognized the maximum deuterium enrichment in a Solar System material, and ion-molecule reactions seem to be the only feasible route to major fractionations. In dark clouds the greatest D enrichments (103-104 compared to SMOW) are observed for HCO, isocyanides and cyanides25, the latter being enriched by exchange reactions with CH2D+ or H2D+. The D enrichment in water in interstellar clouds is difficult to measure; Geiss and Reeves suggest a D content 50 times that of terrestrial water but recognize they are possibly an order of magnitude in error. If the heavy hydrogen in Bishunpur and Semarkona is confined to a specific fraction as seems to be the case in Chainpur7, and indeed exists as water, it is not inconceivable that a practically 'pure' sample of interstellar water has survived into the Solar System. It could be just as important to recognize that the heavy hydrogen is associated with some polymeric carbonaceous phase which condensed from deuterium enriched species. Unequilibrated ordinary chondrites have up to 0.5 wt% 5, which as yet is substantially uncharacterized.

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## Production of NO and N<sub>2</sub>O by soil nitrifying bacteria

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The composition of the atmosphere is influenced both directly and indirectly by biological activity. Evidence is presented here to suggest that nitrification in soil is a potentially significant source of both NO and N2O. Between 0.3 and 10% of the ammonium oxidized by cultures of the soil bacterium Nitrosomonas europaea is converted to these gases. The global source for NO associated with nitrification could be as large as 15×10<sup>4</sup> tonnes N yr<sup>-1</sup>, with a source for N<sub>2</sub>O of 5-10×10<sup>4</sup> tonnes Nyr-1. Nitric oxide has a key role in tropospheric chemistry, participating in a complex set of reactions regulating OH and O<sub>1</sub> (refs 1.2). Nitrous oxide is a dominant source of stratospheric NO (refs 3-5) and has a significant infinence on climate'.

Table 1 Relative rates for NO, N2O and NO2 production by cultures of Nitrosomonas europaea

Oxygen concentration (% v/v)	$\frac{NO/N_2O}{\binom{mol\ N}{mol\ N}}$	$ \frac{N_2O/NO_2^-}{\left(\% \frac{\text{mol } N}{\text{mol } N}\right)} $	$\frac{NO/NO_{2}^{-}}{\left(\% \frac{\text{mol } N}{\text{mol } N}\right)}$	No. of replicates
0.5	$5.1 \pm 3$	$0.9 \pm 0.3$ *	$3.8 \pm 1.2$	2
0.5		$2.5 \pm 0.1$		3
1.0		$0.9 \pm 0.1$	-	3
1.3	1.4†		$1.25 \pm 0.2$	2
5.0		$0.15 \pm 0.02$		3
10.0		$0.16 \pm 0.01$	*****	3
21.0		· —	$0.13 \pm 0.1$	2
21.0	$1.15 \pm 0.15$	$0.47 \pm 0.13$	$0.51 \pm 0.1$	2

Ratios are given as mol % for N2O and NO relative to NO2, but as mol/mol for NO relative to N2O.

\* Production of N2O increased relative to NO2 in this run, from 0.5% initially to 1-2% at the end.

† Estimated from  $N_2O/NO_2^-$  at 1%  $O_2$  and  $NO/NO_2^-$  at 1.3%  $O_2$ .

Enzyme preparations derived from nitrifying bacteria are known to produce trace quantities of NO and N2O (refs 7,8) and release of these gases has also been observed from dense cultures. Goreau et al. 10 investigated rates for production of N<sub>2</sub>O by marine nitrifying bacteria, grown under a range of oxygen concentrations from 0.5 to 20%. The yield for N<sub>2</sub>O, defined as the ratio of N<sub>2</sub>O produced relative to NH<sup>+</sup> oxidized, was about 0.003 mol N N<sub>2</sub>O per mol NH<sub>4</sub> at O<sub>2</sub> concentrations above 2%. The yield rose sharply at lower concentrations of O2, reaching 0.1 for an O<sub>2</sub> concentration of 0.5%. The investigation of Goreau et al.<sup>10</sup> is extended here with studies of a soil nitrifier, N. europaea.

Cells from actively growing cultures were inoculated into 500-ml flasks containing 300 ml of aqueous growth medium<sup>1</sup> with 25 mmol NH<sub>4</sub>. The initial density of cells was  $\sim 10^6$  cm<sup>-3</sup>. The medium was stirred continuously, purged by a stream of gas consisting of N<sub>2</sub> and CO<sub>2</sub> (300 p.p.m.), with predetermined concentrations of O<sub>2</sub>. Temperature was maintained at 25 °C, with pH buffered at 7.5.

Flushing time of the flasks was fixed in the range 4-10 min, sufficient to ensure equilibrium between liquid medium and gaseous headspace. Medium was sampled periodically and analysed for NO<sub>2</sub> and cell numbers (see ref. 10). Concentrations of nitrous oxide and oxygen in the outflow were measured using gas chromatography with electron capture and thermal conductivity detection respectively<sup>11</sup>. Nitric oxide was measured using a chemiluminescence detector, with procedures designed to eliminate interference from other gases 12. Production of N<sub>2</sub>O was measured at  $O_2$  levels of 20, 10, 5, 1 and 0.5%. Production of NO was determined for  $O_2$  concentrations of 20, 1.3 and 0.5%.

Experimental results are presented in Fig. 1 and Table 1. Production rates for N<sub>2</sub>O, NO and NO<sub>2</sub> were closely parallel, as observed previously <sup>10</sup> for NO<sub>2</sub> and N<sub>2</sub>O. Cultures produced N<sub>2</sub>O and NO with uniform ratios relative to NO<sub>2</sub> at O<sub>2</sub> levels above 1% (Fig. 1a, c). Replicate flasks gave consistent results, with variability largest at low O2. The rate for evolution of NO correlated strongly with that for N<sub>2</sub>O (see Fig. 1d). Measurements of cell numbers showed that cell division did not occur in cultures with  $O_2$  equal to 20%, and production of  $NO_2^-$ , NO and  $N_2O$  were constant with time in these experiments (see Fig. 1a). Active cell division occurred in all other experiments, with doubling times of  $0.8 \pm 0.2$  days. Yields for NO and N<sub>2</sub>O were constant relative to NO<sub>2</sub>, independent of whether or not cells were dividing. An exception occurred at 0.5% O2, where the yield for N<sub>2</sub>O increased with time even though the yield for NO remained constant.

Results for NO and N<sub>2</sub>O are given in Table 1 at various oxygen concentrations. Yields for both gases rose markedly at O<sub>2</sub> concentrations below 2%. The ratio of NO production relative to N2O also rose as O2 decreased, with a median value of ~1.5 mol N NO per mol N N2O.

Two experiments were performed which indicated a metabolic origin for the bulk of NO observed in the present work. First, sterile culture medium was spiked with  $NO_2^-$  to a concentration above that in the active cultures. A small transient pulse of NO was observed but emission terminated within minutes. Second, an actively growing culture of *N. europaea* was killed by addition of 1 ml saturated solution of a potent respiratory inhibitor,  $HgCl_2$ . The NO signal declined promptly, falling within 50 min to 10% of the value observed before addition of  $HgCl_2$ . Decay of NO followed the time course expected from flushing of the experimental apparatus, with negligible (<10%) abiotic production. The behaviour was similar to that observed earlier for  $N_2O$  (ref. 10).

The concentration, C (cm<sup>-3</sup>), of NO or N<sub>2</sub>O measured at the outlet of the apparatus may be written in the form

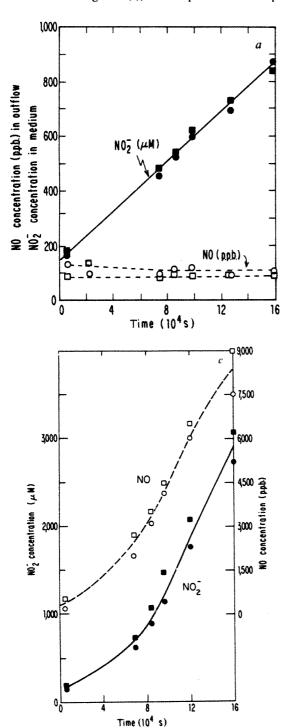
$$C = Pt/(1 + Lt)$$

where t is the flushing time (s), P is the production rate per unit

volume of the apparatus (cm<sup>-3</sup>s<sup>-1</sup>) and L is a loss frequency (s<sup>-1</sup>) introduced to account for possible loss in the medium, in the head space or on the walls of the apparatus. Figure 2 shows a plot of CV/t, the mass flux from the apparatus, as a function of t (where V is the volume of the apparatus, t = V/(flow rate)). This quantity should be independent of t for  $Lt \ll 1$  and should vary as  $t^{-1}$  for  $Lt \gg 1$ . The results in Fig. 2 suggest that losses of NO are negligible in the present system, that  $L^{-1} \gg 4$  min. Loss of N<sub>2</sub>O is similarly small<sup>10</sup>.

Nitrification is thought to provide a major source for atmosphere.

Nitrification is thought to provide a major source for atmospheric  $N_2O$  (refs 10, 13, 14). The global source of  $N_2O$ , known from considerations of atmospheric chemistry<sup>15</sup>, is  $10\times10^6$  tonnes N yr<sup>-1</sup>. If we assume that this source is associated primarily with nitrification and adopt relative production rates indicated in Table 1, we may estimate that the corresponding source of NO could be as large as  $15\times10^6$  tonnes N yr<sup>-1</sup>. There may be sources of  $N_2O$  in addition to nitrification, for example, denitrification<sup>16</sup> and lightning<sup>17</sup>. Our estimate for the



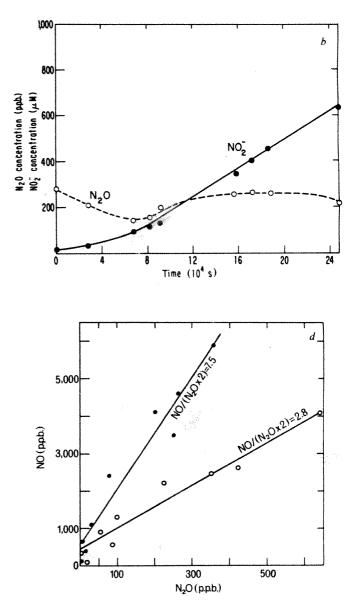


Fig. 1 Measured concentrations of NO,  $N_2O$  and  $NO_2^-$  in selected experiments with N. europeaea at different oxygen tensions. Gas concentrations were measured in the outflow of the apparatus and are proportional to the production rates by the cultures. Nitrite accumulated in the medium and hence measured concentrations must be differentiated to obtain production rates. a, NO and  $NO_2^-$  at 20% O<sub>2</sub>, squares and circles represent replicate cultures; b,  $N_2O$  and  $NO_2^-$  at 1% O<sub>2</sub>; c, NO and  $NO_2^-$  at 1.3% O<sub>2</sub>; d,  $N_2O$  and NO at 0.5% O<sub>2</sub>.

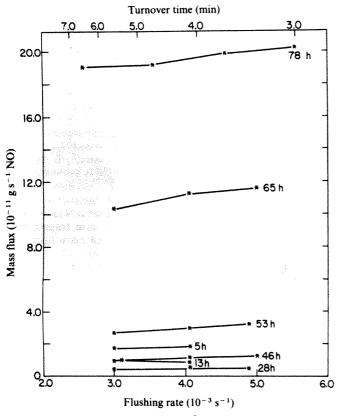


Fig. 2 The mass flux of NO (g NO s<sup>-1</sup>) is plotted as a function of gas flow rate through the apparatus, at various times after inoculation. The flushing rate is defined as (flow rate)/(head space volume). For 0-46 h, purge gas contained 20% O2, the cells did not divide and NO was produced at low rate. Reduction of O2 to 0.5% induced almost immediate increase in production of NO, NO2 and bacterial cells. At both oxygen levels the mass flux seems to be independent of flow rate, implying negligible loss of NO in the apparatus (see text).

nitrification source of NO would be lowered accordingly. Also, NO could be removed by heterogeneous processes before release to the atmosphere (see ref. 12).

Galbally and Roy<sup>18</sup> observed fluxes of NO with an average value of  $2 \times 10^{-12}$  kg N m<sup>-2</sup>s<sup>-1</sup> from soils in Australia. They estimated a corresponding global source of 10×10<sup>6</sup> tonnes N yr<sup>-1</sup>, similar to the value derived here. Our data are consistent with the view that nitrifying bacteria could produce a substantial fraction of the NO observed by Galbally and Roy18, although there could also be production by dentrification 19 or by abiotic reactions involving NO<sub>2</sub> (refs 20,21).

The source of NO derived here is similar in magnitude to production by combustion<sup>2,22</sup>, photooxidation of NH<sub>3</sub> (ref. 23), decomposition of stratospheric N<sub>2</sub>O (refs 15,22) and lightning (ref. 24). The lifetime of fixed nitrogen species in the atmosphere is rather brief<sup>2,25</sup>. It follows that nitrification in soils could exercise a major influence on the NO, budget of the troposphere, with particular significance for regions remote from

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### Structure sensitivity in the iron single-crystal catalysed synthesis of ammonia

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There have been many investigations of the kinetics of the iron-catalysed synthesis of ammonia and the details of the mechanism of its reaction<sup>1-3</sup>. However, we report here the first direct and, therefore, unambiguous measurement of the surface structure sensitivity of the iron-catalysed ammonia synthesis reaction using the (111), (100) and (110) crystal faces of iron and operating at pressures approaching those used industrially (20 atmospheres). The Fe(111) face was found to be the most active, the activity ratio being 418:25:1 for the Fe(111), Fe(100) and Fe(110) planes. We have also observed the formation of a strongly bound nitrogen species after the reaction, in the presence of which ammonia continues to be produced.

The apparatus<sup>4</sup> consisted of a stainless steel ultrahigh vacuum chamber equipped with a four-grid retarding field analyser for Auger and low-energy electron diffraction measurements of the surface composition and structure, a residual gas analyser, an ion gun for specimen cleaning and a high-pressure cell connected to an external loop. Reactant and product gases at a total pressure of 20 atmospheres were continuously circulated over the heated iron crystal specimen by a positive displacement pump; samples, periodically withdrawn using a gas chromatograph type sampling valve, were passed by a nitrogen carrier through a photoionization detector to determine the amount of ammonia in the loop. Ammonia was the only species present which could be detected by the photoionization detector obviating the need for a separating column.

Circular iron specimens with crystal faces (111), (110) and (100) were spark-cut from a high-purity (4N) single-crystal rod and diamond polished, the final dimensions being ~6 mm diameter and 1 mm thickness. Sulphur and carbon were found to be the principal contaminants after this procedure, but could be removed by several days of heating at 800°C in 1 atmosphere of hydrogen, followed by many cycles of argon ion bombardment and annealing at 700 °C. To minimize the catalysis occurring on ill-defined iron surfaces, platinum foil was spot-welded to the rough edges of the specimens.

Results of measurement of structure sensitivity are shown in Table 1 from which it can be seen that the (111) surface of iron catalyses the synthesis of ammonia from hydrogen and nitrogen at least 418 times faster than the ordered (110) surface and 16 times faster than the ordered (100) surface. Our result is consistent with the low pressure chemisorption studies of Ertl and co-workers2 where it was reported that the rate of dissociative chemisorption of nitrogen is greatest on the (111) plane of iron

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Table 1 Surface structure sensitivity of ammonia synthesis

Catalyst surface	Area (cm²)	Rate* (mols NH <sub>3</sub> cm <sup>-2</sup> s <sup>-1</sup> )
Fe(111)	0.63	$4.6 \times 10^{-8}$
Fe(100), ordered	0.80	$2.8 \times 10^{-9}$
Fe(110), ordered	0.57	0†
Fe(100), disordered‡	0.80	$4.5 \times 10^{-9}$
Fe(110), disordered‡	0.57	$9.7 \times 10^{-10}$

- \* Total pressure, 20 atm.;  $P_{\rm H_2}/P_{\rm N_2}=3/1$ ; temperature = 798 K. † Detection limit ~1.1×10<sup>10</sup> mol NH<sub>3</sub> cm<sup>-2</sup> s<sup>-1</sup>. ‡ Argon ion bombardment  $(1.5\times10^{-5})$  A cm<sup>-2</sup> for 10 min at 500 V).

and least on the (110) plane, and also with previous suggestions<sup>1-3,5</sup> that this step is rate-determining in the ammonia synthesis reaction.

The (111) plane of iron contains both seven-coordinated  $(C_7)$ and four-coordinated (C<sub>4</sub>) atoms, whereas the (110) and (100) planes contain only six-coordinated (C<sub>6</sub>) and C<sub>4</sub> atoms, respectively. The large variation in rates of ammonia synthesis on the various planes (Table 1) and the anomalously high catalytic activity that we have also detected on the (110) and (100) planes when deliberately disordered by ion bombardment, support the proposals of Brill<sup>6</sup> and others<sup>3,7</sup> regarding the importance of clusters and C7 iron atoms as components of the active site on the catalyst surface.

Although bulk nitrides of iron are thermodynamically unstable in the conditions of these experiments, we have observed the existence of a long-lived, strongly-bound nitrogen species after the synthesis reaction on both Fe(111) and Fe(100) crystal faces in the presence of which ammonia continued to be produced at an undiminished rate. The catalytic significance of this surprising observation has yet to be determined.

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## An oceanic carbonatite volcano on Santiago, Cape Verde Islands

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Carbonatite volcanoes are rare, the only active one being at Oldoinyo Lengai in northern Tanzania<sup>1</sup> although a few dead volcanic centres with recognizable carbonatitic extrusive materials are known elsewhere in Africa2-6 and one in Germany<sup>7</sup>. During an expedition in 1980 to the Cape Verde Islands, in the Central Atlantic Ocean 500 km west of Senegal, the eroded remains of a carbonatite volcano were discovered on the island of Santiago just north of Tarrafal at Arruela (Fig. 1). This, the first example of an oceanic carbonatitic volcanic pyroclastic structure, shows typical carbonatite mineralogy and geochemistry, and demonstrates that carbonatite magmas can be generated in the oceanic lithosphere as well as in the continents.

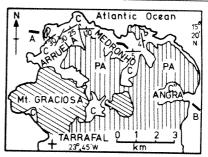


Fig. 1 Geological sketch map of northern Santiago (after Serralheiro<sup>9</sup>). S, Phonolite (youngest lavas); III, Pliocene plateau basalts of Pico da Antonia Formation (PA); ©, intrusive, pyroclastic and epiclastic carbonatitic lithic tuffs. C" is explained in Fig. 2.

An oceanic character for the Cape Verde Islands region seems the most probable, although the possibility of a fragment of continental crust existing in the area cannot be totally discounted. However, the position of these volcanic islands some 400 km west of the westernmost parts of the West African continental shelf astride the Mesozoic oceanic geomagnetic anomalies<sup>8,9</sup>, the mid-oceanic ridge basalt geochemical character of the pillow basalts of the region 10 and the fact that the islands have apparently grown from the oceanic floor, leave little doubt as to their oceanic setting.

Carbonatitic magmatism has been said to characterize intraplate continental crust<sup>5</sup>, but this discovery of a carbonatite volcano and therefore of undoubted carbonatite magmatism demonstrates that such magmas can form in oceanic lithosphere. The intrusive carbonatitic equivalents, together with ijolites, on most of the other Cape Verde Islands (and some of the Canary Islands) suggest that intraplate oceanic carbonatitic magmatism is not rare; in fact it is commonplace in all oceanic islands with long geological histories so far investigated.

Only the basal wreck of the former pyroclastic cone on Santiago remains (Fig. 2), and it comprises carbonatitic and nephelinitic subaerial tuffs pierced by carbonatite and nephelinite dykes and veins. In every petrological feature, apart from the absence of natrocarbonatite lava, the structure and petrology are exactly similar to those at Oldoinyo Lengai volcano. The greater part of the cone seems to have been planed off by erosion, and much of the material redeposited as shallow marine epiclastic carbonatitic conglomerates, siltstones and calcilutites. These are covered by bioclastic calcarenites and plateau basalts.

Beneath the erosion level, exposures in the western sea-cliffs reveal great thicknesses of massive pale-coloured carbonatecemented agglomerates, ashes and tuffs which carry an assortment of coarse-grained, fine, dark, light-coloured, big (1m) and small blocks. Elsewhere the rocks are better bedded with occasional large-scale low-angled cross-bedding, generally dipping seawards. They and the associated agglomerates are calcareous, and all abound with biotite flakes and lithic fragments including apatite-sövite, ijolite, apatite-pyroxenite and olivine-poor nephelinite.

The unique carbonatitic character of the sub-aerial ochrecoloured tuffs which make up the bulk of this former volcanic structure, is not immediately apparent in the field, and had not been recognized by previous workers<sup>11,12</sup>. The microscope reveals that the tuffs are 95% or more of rounded grains of calcite crystallites, each grain usually having a brown-stained core. This texture is similar to that seen in carbonatitic tuffs in East Africa. Some horizons include lapillistone containing spherical lava tears with quench textures identical to those described recently from Kaiserstuhl<sup>7</sup>. Interleaved with the carbonatitic tuffs and agglomerates are large masses of pale grey-green unbedded clays up to 5 m thick. As the clays carry abundant ijolite and nephelinite clasts, these clays are interpreted as altered nephelinitic tuffs and ashes. The assemblage is identical to that at Oldoinyo Lengai<sup>1</sup>, and it all points to a mainly pyroclastic mechanism of eruption with much of the lava being emitted as droplets perhaps during nuée ardente eruptions.

Fig. 2 Cross-section along line A-B in Fig. 1 showing the eroded remains of the carbonatitic and pyroclastic volcanic cone (CA) penetrated carbonatite adventive plugs (C') and dykes, unconformably (shown schematically) overlain by



carbonatitic epiclastic beds (CB) and calcarenites (dotted); above are Pico da Antonia basalts (PA). At Angra is a carbonatite-ijolite-syenite-breccia plug (C") which intrudes basalt pillow lavas (P), and is overlain unconformably by PA basalts.

Penetrating the tuffs are a great variety of dykes and plugs. Several tuff dykes, 0.1-3.0 m wide, carry abundant ijolite fragments, some as cored autoliths with nephelinitic coatings. Other dykes which run in all directions are often only 0.2-0.5 m wide and include olivine-poor nephelinites (with nepheline, aegirineaugite, melanite-garnet, perovskite, apatite, analcime and some phlogopite) and carbonatites. One carbonatite dyke 0.2 m wide made up entirely of carbonate revealed, under the microscope, discontinuous pieces of convoluted flow texture of deformed ovoids, and appears to represent a flowed and fragmented carbonatitic pumice which must have been emplaced in a fissure not far below a point of extrusion.

Intrusive plugs, one up to 15 m diameter<sup>12</sup>, of flow-banded dolomitic carbonatite brecciate their way through the bedded tuffs. Also penetrating the tuffs are vertical, roughly circular haematitic patches 1-3 m across, apparently representing paths of fumarolic fluids passing upwards through the ochreous tuffs.

An 87Sr/86Sr determination of the Arruela plug carbonatite (at the Université de Clermont-Ferrand) gave 0.7040 which is close to earlier determinations of other Cape Verde and Canary Island carbonatites<sup>13,14</sup>. This confirms the igneous character of these carbonatite plugs. Preliminary X-ray fluorescence analysis (H. Furnes at Bergen University) for the trace element content of the carbonatites (Table 1) shows high contents of the incompatible elements which is typical of true carbonatites<sup>6</sup>. They are further noteworthy for the unusually high Zn content and the strong rare-earth enrichment in the intrusive carbonatites

A period of sub-aerial followed by marine erosion seems to have decapitated this pyroclastic volcano, leaving it buried beneath 10-100 m of epiclastic sediments largely composed of detritus derived from the destruction of the volcano. Conglomerates and grits fill hollows in the underlying tuffs. The dips are variable and usually low (0°-20°) except where now seen in some slipped sections along the cliffs. Some beds are well stratified fine siltstones, some with graded bedding, others with cross-lamination. There are moulds of large marine gastropods. In further contrast to the volcanic tuffs below, these beds are rarely cut by dykes and then only by basaltic ones, and are quite well sorted. Clasts of ijolite, pyroxenite and nephelinite up to 0.1 m across are common and biotite flakes abound. Under the microscope these epiclastic siltstones and grits are seen to be composed of abundant grains of carbonatite, recognizably the

Table 1 Trace elements in Cape Verde carbonatitic rocks, preliminary results in p.p.m.

	1	2	3	4	5	6
V	136	140	200	89	435	114
Żn	240	304	245	309	617	758
Rb	6	7	7	10	14	13
Sr	490	968	1,176	6,210	4,500	4.320
Y	70	72	102	37	205	137
Nb	72	41	427	14	195	43
La	683	900	1,427	2,790	5,850	8,040
Ce	921	1,175	2,028	3,373	6,913	10,043
Nd	299	358	862	914	2,056	2,791

Columns 1 and 2: dolomitic carbonatitic intrusive tuffs of the Arruela plug. Column 3: carbonatitic tuff dyke cutting tuffs near the Arruela plug. Column 4: apatite-carbonatite, recrystallized block in carbonatitic breccia at Angra. Column 5: metabasalt veined and impregnated by carbonatite, Angra. Column 6: metabasalt metasomatized by carbonatite, Angra.

same as those in the underlying tuffs, and with detrital apatite, aegirine, garnet, perovskite and chert.

In places along the erosional unconformity with the overlying Pico da Antonia Formation alkali basalts, these lenticular epiclastic sediments are strongly reddened. There is also a concentration of ijolite, nephelinite, basalt and red carbonatite clasts in the conglomerate marking this unconformity. In the Ponta Moreia area, marine bioclastic calcarenites, a few metres thick and containing oncolites, usually lie immediately under the basalts.

It is estimated that the exposed area of the carbonatitic pyroclastic volcano remnant exceeds 10 km<sup>2</sup>, and any envisaged reconstruction of a volcano on this site produces a structure very similar to that at Oldoinyo Lengai in northern Tanzania.

The main conduit of the volcano is unknown. The vent might be sited centrally in the northern part of Santiago and would then be hidden beneath the later basalt and phonolite lavas. But it is more likely that the intrusive ijolites, carbonatites and breccias at Angra, presently standing nearly 200 m above sea level and penetrating old pillow lavas, mark the site of the vent-plug. The age of the volcano is not known except that it is older than the Pico da Antonia basalts which previous workers estimate are Pliocene-Miocene<sup>12</sup>. K-Ar dating<sup>15</sup> gave 4-5 Myr for the basalts, and 8.5 and 9.8 Myr for intrusive carbonatites elsewhere in Santiago.

Carbonatitic volcanism must therefore no longer be considered as restricted to continental regions. This oceanic intraplate occurrence demonstrates that carbonatite magma can form in suboceanic as well as subcontinental lithospheric mantle. A further implication would seem to be that whilst small ocean islands such as St Helena represent small developments of alkaline magmatism, the bigger archipelagos such as the Cape Verdes seem to be the result of more extensive alkaline magmatism which can produce more extreme alkaline compositions. This probably reflects greater mantle metasomatism in the source regions. Mantle heterogeneity has been widely advocated recently 16, but such extremes beneath oceans have not been suggested before.

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## Platinum and palladium minerals in upper mantle-derived lherzolites

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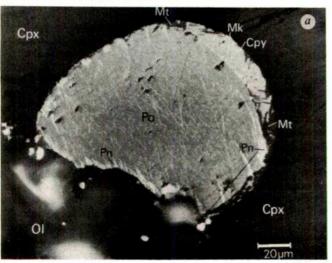
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Spinel Iherzolite xenoliths in basanites from the Newer Volcanics of Western Victoria, Australia contain sulphide inclusions along grain boundaries and within fractures in the silicates. The inclusions are complex intergrowths of pentlandite, pyrrhotite and chalcopyrite formed by the breakdown of Fe-Ni monosulphide solid solutions. During this process discrete grains of a platinum sulphide and a palladium stannide were exsolved from the pentlandite. We show here that this is the first evidence for sulphide fluids rich in platinum group

elements in the upper mantle.

During an investigation of the distribution of Au, Pd and Ir in mantle-derived spinel lherzolites from basanites of the Newer Volcanics of Western Victoria, Australia2, it was discovered that the bulk (60-80%) of these metals are not held in solid solution in any of the major silicate or oxide phases but are concentrated in the intergranular regions1. The intergranular phases consist of serpentines, magnetite, goethite and sulphides which are located along grain boundaries and within fractures in the silicates. Textural evidence indicates that the sulphides have been introduced into the xenoliths along these microfractures. The sulphides were not introduced into the xenoliths during transport as the host basanite lacks such materials, is undersaturated in sulphur and is poor in Au, Ir, Pd1. We consider that the sulphides were added to the xenoliths in the mantle and represent an immiscible sulphide melt formed during a partial melting episode before that which generated the host lava which merely transports the xenoliths to the Earth's surface<sup>1</sup>. In view of the chalcophilic properties of the platinum group elements<sup>3</sup>, it was proposed that these sulphides might be the major host of the intergranular platinum group elements1. To investigate this



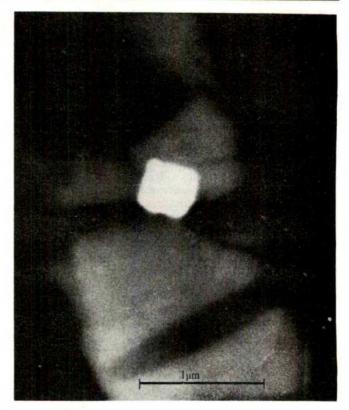


Fig. 2 Back-scattered eletron photograph obtained using a Robinson detector coupled to a scanning electron microscope. The euhedral phase showing strong atomic number contrast (white) is platinum sulphide set in a pentlandite host (grey). Black areas are cleavages or fractures.

hypothesis we examined the sulphides using scanning electron microscopy and discovered discrete grains of platinum group minerals.

Figure 1a shows a typical ovoid sulphide inclusion and illustrates the complexity of the sulphide intergrowths. The bulk of the globule consists of pyrrhotite with exsolved flames of pentalandite, this assemblage being mantled by chalcopyrite containing exsolved mackinawite. The chalcopyrite is replaced along cleavages by magnetite.

Figure 1b shows another sulphide globule consisting of pentlandite which has been replaced by magnetite. Within this

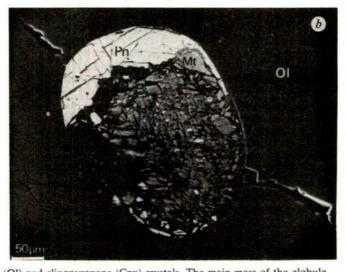
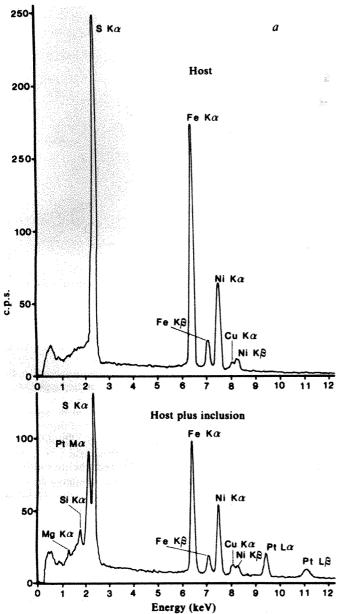


Fig. 1 a, Composite sulphide globule at the contact between olivine (OI) and clinopyroxene (Cpx) crystals. The main mass of the globule consists of pyrrhotite (Po) containing exsolved pentlandite (Pn). This is mantled by chalcopyrite (Cpy). Thin lamellae of mackinawite (Mk) occur within the chalocopyrite which is also replaced along cleavages by magnetite (Mt). This photomicrograph illustrates the incipient replacement of sulphides by oxides. Reflected light photograph of polished thin section. b, Sulphide globule located within a fracture in olivine consisting of pentlandite and magnetite. This was originally an entire sulphide globule similar to that shown in a. The black areas mark plucked sulphide or oxide. Reflected light photograph of polished section



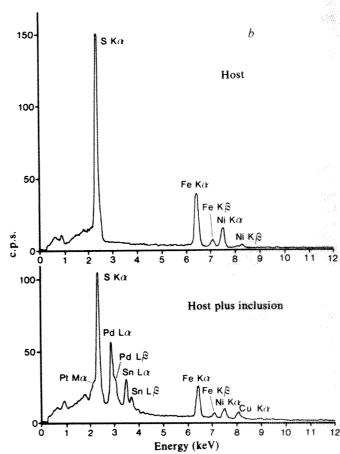


Fig. 3 X-ray energy dispersive spectra of host pentlandite (upper) and of host plus inclusion (lower). a, Inclusion is platinum sulphide;
b, inclusion is palladium stannide.

globule we located the discrete Pt-mineral described below. The original mineralogy of the globule before magnetite replacement was probably similar to that of the inclusion shown in Fig. 1a.

Platinum group minerals were located using a Robinson back-scattered electron detector coupled to a scanning electron microscope<sup>4</sup>. This type of imaging enhances the atomic number constrast for back-scattered electrons from matrices of differing average atomic number and is ideally suited for the detection of platinum group minerals of high atomic number in a Fe-Ni-sulphide matrix. Figure 2 illustrates the excellent atomic number contrast exhibited between a Pt-mineral and its pentlandite host.

The inclusion is too small  $(0.5 \,\mu\text{m})$  to extract for X-ray diffraction identification and for quantitative in situ electron microprobe analysis, as the excitation area produced by the electron beam is larger than the inclusion, resulting in excitation of the host. Figure 3a thus shows X-ray spectra of the pentlandite host and of the host plus inclusion to demonstrate that the inclusion is a discrete phase. The spectrum of the host plus inclusion contains strong Pt X-ray lines which are lacking in the host alone. The presence of minor peaks attributable to Mg and Si indicates fluorescence of intergranular serpentine or of the olivine which hosts the sulphide inclusion. The mineral lacks As, Sb, Bi, Pd and Sn, any S present is masked by the S peaks of the

pentlandite. From this qualitative analysis we believe the inclusion to be a platinum sulphide, probably cooperite (PtS).

Examination of other sulphide globules resulted in the discovery of several anhedral grains of a palladium mineral in a pentlandite host. Figure 3b shows X-ray spectra for the host and the inclusion plus host. The presence of strong X-ray lines of Pd and Sn together with only a weak Pt line and the absence of As, Sb, Bi indicates that the mineral is a palladium stannide. Unfortunately, we were not able to analyse this phase quantitatively. The mineral could possibly be paolovite  $(Pd_2Sn)$ , atokite  $(Pd_2Sn)$  or stannopalladinite  $(\alpha-Pd_3Sn_2)$ .

Sulphide globules containing complex Cu-Ni-Fe sulphide intergrowths similar to those described above have been noted to occur in spinel lherzolites and eclogites<sup>5-7</sup>. However, to our knowledge this is the first occurrence of discrete Pt and Pd minerals in such an upper mantle xenolithic paragenesis.

Studies of magmatic nickel-platinum group element ore deposits such as those associated with the Sudbury and Bushveld intrusions coupled with experimental studies of Cu-Ni-Fe sulphide phase quilibria have demonstrated that the precursors to the pentlandite-pyrrhotite-chalcopyrite intergrowths are Fe-Ni-monosulphide solid solutions which crystallized from a sulphide liquid initially formed by liquid immiscibility from a sulphur saturated basic silicate melt<sup>8,9</sup>. The originally homogeneous monosulphide undergoes a variety of subsolidus

breakdown reactions as it cools from magmatic temperatures (~1,000°C). For example, pentlandite only becomes stable below 610°C and the pentlandite-pyrrhotite intergrowths probably develop below 300°C (ref. 6). Platinum group minerals in such parageneses occur as discrete grains in sulphide host and exhibit textural relations similar to those observed in the spinel lherzolites. Cabri and Laflamme<sup>10</sup> proposed that most of the platinum group minerals in such ores must also have originated by sub-solidus exsolution from a platinum group element-rich sulphide. Accordingly we believe that the discrete Pt and Pd minerals noted above have exsolved from pentlandite at low temperatures, the pentlandite itself having previously formed by the low temperature breakdown of Fe-Ni monosulphide solid solution. The initial sulphide liquid was introduced into the lherzolite within the mantle and in those conditions would have crystallized to a homogeneous platinum group element-rich monosulphide solid solution. Sub solidus breakdown probably occurred after incorporation into the transporting magma during cooling of the lava subsequent to eruption.

Speculation concerning the source of the sulphide liquids is beyond the scope of this note, but the above observations demonstrate that platinum group element-rich sulphide liquids are present in the upper mantle. This conclusion has important consequences with regard to the geochemistry of the platinum metals in general and in particular to their distribution between the Earth's core and mantle and to the possibilities of the addition of magmatic sulphide liquids to lherzolite within the upper mantle.

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## Cross-polarization <sup>13</sup>C-NMR spectroscopy with 'magic angle' spinning characterizes organic matter in whole soils

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The limitations of <sup>13</sup>C cross-polarization (CP) NMR spectroscopy with 'magic angle' spinning to characterize the organic matter in whole soils have recently been discussed1: resonances from rotor materials and from spinning sidebands prevented a complete analysis of the types and forms of carbon in whole soils. We now report experiments in which these problems have been overcome. The results show that carboxylic, aromatic, acetal, alkoxy and alkyl carbon are present in soils and they allow approximate estimates of various carbon types to be made without previous extraction from the soil.

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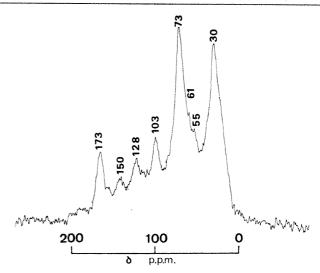


Fig. 1 CP-MASS spectrum of Mapourika soil. Spectrum was obtained with a contact time of 1 ms and a recycle time of 0.3 s; 10<sup>5</sup> transients were collected.

There have been no adequate techniques for characterizing soil organic compounds in the solid state. Moreover, the limited solubility of these materials, even in strongly alkaline media, makes studies on extractable material of limited applicability. CP-13C-NMR spectroscopy could provide qualitative information about the fraction of carbon which is aromatic, the aromaticity  $(f_a)$  of whole soils<sup>2</sup>, but detailed information on the chemical structure of in situ organic matter is hidden because the linewidths of CP-13C-NMR signals in solids are of the order of 200 p.p.m. due to chemical shift anisotropy. Spinning the sample at an angle of 54° 44' to the applied field (the 'magic angle' sample spinning technique, MASS) reduces linewidths to a magnitude comparable with that obtained for species in solution. Unfortunately, the low carbon content of soils precludes the use of conventional rotor materials (usually deuteopolymethylmethacrylate) for obtaining spectra of whole soils<sup>1,2</sup> have used a rotor consisting of a barrel of boron nitride with a seat of Kel F (K.W.Z., unpublished work), and sufficient spinning speeds (~3.5 kHz at 25.1 MHz <sup>13</sup>C frequency) to remove sidebands. Some characteristics of the soils used are given in Table 1 (more detailed information is available elsewhere<sup>4-7</sup>). Five soils have been investigated in this initial study, two young soils (age <400 yr) and three older soils (age >11,000 yr). Two

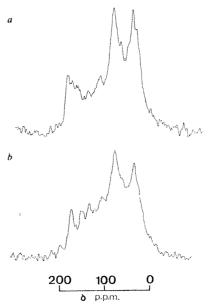


Fig. 2 CP-MASS spectra of a, Franz X and b, Ikamatu soil. Spectra were collected in similar conditions as Mapourika soil.

Table 1 Characteristics of soils								
Soil	Horizon	Age (yr)	pH (1:2.5 with H <sub>2</sub> O)	C (%)	Parent material	New Zealand classification		
Hokitika	A	300	5.5	2.8	Glacial	Recent		
Ikamatu	A	14,000	4.6	9.8	gravels Glacial gravels	Lowland yellow brown carth		
Mapourika	A	12,000	4.2	7.9	Glacial moraine	Gley podzol		
Franz X	A	350	5.2	6 6	Glacial moraine	Gley podzol		
Tirau	Α	********	5.7	10.5	Rhyolitic ash	Central yellow brown loam		

of the soils (Hokitika and Ikamatu) form part of the Reefton chronosequence and two others (Mapourika and Franz X) are from the Franz Josef chronosequence. Both chronosequences are in the South Island, New Zealand.

Figure 1 shows the CP-MASS- $^{13}$ C-NMR spectrum of Mapourika soil.  $^{13}$ C resonances can be assigned if it is assumed that the isotopic chemical shifts ( $\delta$ ) in such solids are not significantly different (that is  $\pm 2$ -3 p.p.m.) from those in solution. The spectrum can be divided into five regions:  $\delta$  10–50 p.p.m. (alkyl carbon),  $\delta$  50–100 p.p.m. (O-alkyl carbon),  $\delta$  100–110 p.p.m. (acetyl carbon),  $\delta$  110–160 p.p.m. (alkene and aromatic carbon) and  $\delta$  160–200 p.pm. (carbonyl carbon). The aromatic region consists of two distinct groups of resonances centred around  $\delta$  128 p.p.m. and 150 p.p.m. The latter most probably arises largely from aromatic carbons next to oxygen (phenols and furans). The former is protonated carbon plus substituted carbons of aryl carboxylic acids.

The proportions of various carbon types present in the soil determined by integrating the six regions of the spectrum in Fig. 1 are given in Table 2. These values are only approximate estimates for several reasons. First, because resonances from the various carbon types are not fully resolved, it is not possible to integrate the spectra with great accuracy. Second, it is not possible to differentiate between carbons which may be unusually shifted upfield or downfield from their respective common chemical shifts. Third and most important, the cross-polarization process does not necessarily give quantitative data. It must be established that the proton population relaxes with a uniform time constant  $(T_{1p})$  and that sufficient time delays between repeating pulse sequences allow total relaxation of protons. The rate of cross-polarization depends on the proximity of protons to carbons, hence the contact time between carbons and protons must be optimized to ensure all carbon types are polarized to an equal extent.

Although  $T_{1p}$  values in soils are short  $(<10 \text{ ms})^2$  there is some evidence that results obtained by CP-MASS- $^{13}$ C-NMR are quantitative. In CP experiments maximum enhancement of different carbon types occurs at contact times and recycle times

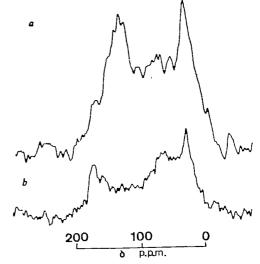


Fig. 3 CP-MASS spectra of a, Hokitika and b, Tirau soil. Spectra were collected in similar conditions as Mapourika soil.

of magnitudes used in the present experiments<sup>2</sup>. In addition, aromaticities of humic substances measured by CP, CP-MASS and conventional solution NMR are similar<sup>8,9</sup>, and CP-MASS has been widely used in the interpretation of closely related coal samples which have similar relaxation rates<sup>10-12</sup>.

Table 2 clearly shows that alkyl carbon is an important component of Mapourika soil. Because the alkyl signal is centred around 30 p.p.m. much of this carbon is polymethylene [(CH<sub>2</sub>)<sub>n</sub>]. The evidence for polymethylene in soils has been reviewed<sup>13</sup>. Aliphatic carbon  $\alpha$  to oxygen is also an important component of the soil; about 35% of the carbon is in this form. Small amounts of acetal carbon are also present. The O-alkyl and acetal carbon probably originate from carbohydrates. The ratio of O-alkyl to acetal carbon is 4:1 which, bearing in mind the approximate nature of the results, is consistent with hexose sugar units such as those in cellulose. About 10% of the carbon in Mapourika soil is carboxylic. This is in excellent agreement with typical values obtained for humic acids by conventional functional group analysis<sup>14</sup>.

It had been assumed that all carboxylic carbons in soil organic matter are attached to aromatic rings, however, Anderson and Russell<sup>15</sup> suggest that polymaleic acid-like molecules are important components of humic substances; that is, many of the carboxylic groups are attached to alkyl groups. The aromaticity (fraction of carbon which is aromatic,  $f_a$ ) of the Mapourika soil is low (~15%). The fraction of aromatic carbon is only slightly higher than the fraction of carboxylic carbon (10%). Much of the aromatic carbon is clearly not attached to carboxylic groups, as a significant proportion of the aromatic carbon is oxygenated or protonated. Hence, our results suggest that many of the carboxylic carbons are bound to aliphatic carbon<sup>15</sup>.

The spectra of the other soils (Figs 2, 3) show that the structure of soil organic matter can vary widely from soil to soil. The

		Table 2	Approximate estimates of carbon types in soils								
	Assignment Chemical shift peak	Alkyl 30	O-Alkyl 73		Acetal 103	ArylH* 128	ı	Aryl-R†‡ 150	<u>f.</u>	COOR§	CO 198
Soil											
Mapourika		0.30	0.35		0.09	0.10		0.05	0.15	0.10	
Ikamatu		0.26	0.33		0.11	0.11		0.08	0 19	0.08	0.03
Hokitika		0.27	•	<b>-0.30 </b>		◀	<b>-0.37</b> -		0.37	0.06	
Franz X		0.33	0.33		0.07	<del></del>	<del></del> 0.17 -	<b>─</b>	0.17	0.10	
Tirau		0.30	←	<b></b> 0.37 <b></b> -		4	-0.23 -	<b>→</b>	0.23	0.10	

<sup>\*</sup> Some substituted carbon of anyl carboxylic acids or phenolic anyl carboxylic acids may occur in this region.

<sup>†</sup> R = O. C.

<sup>‡</sup> Largely aromatic oxygenated carbon.

R = H, metal ion, alkyl.

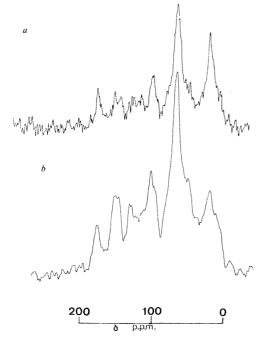


Fig. 4 CP-MASS spectra of soil decomposition products of a, beech and b, pine leaves (needles). Leaves were aged for 2 yr by microbiological decomposition. Detailed experimental data are available elsewhere

degree to which different functional groups can be resolved depends on the preponderance of one carbon type to another. Most of the detail present in the spectrum of Mapourika soil is also present in the spectrum of Ikamatu soil (Fig. 2b) but the greater amounts of acetal and aromatic carbon mean that different carbon types are not so well resolved. The Hokitika soil is a case in point (Fig. 3a); about 37% of the carbon is aromatic and 27% alkyl. The O-alkyl and acetal carbons are not resolved, nor are substituted and unsubstituted aryl carbon resonances.

Even though unsuitable rotors were used, the three soils (Maungatua, Okarito and Te Kopuru) previously investigated by CP-MASS-NMR<sup>3</sup> show enough detail to allow them to be classified with the soils described above. Maungatua humic silt loam is highly aliphatic and similar in organic structure to the Mapourika soil except the O-alkyl and acetal carbon content is much lower. The other two soils (Te Kopuru and Okarito) are of more aromatic character and similar to Ikamatu soil. Nevertheless, these soils also show that the O-alkyl and acetal content can vary considerably<sup>3</sup>.

Figures 1 and 2 show that further, qualitative information is available from CP-MASS-13C-NMR spectra. Mapourika soil shows evidence of carbon resonances at 61 p.p.m., probably due to  $-CH_2OH$  carbon. There is also resonance at  $\delta$  55 p.p.m. which is possibly methoxy carbon. The Ikamatu spectrum (Fig. 2b) offers very tentative evidence for ketone carbon at

Clearly, CP-MASS-13C-NMR has enormous potential in soil science. No other analytical technique reveals such structural detail on the nature and forms of organic carbon. More extensive studies than those reported here may clarify the various processes resulting in soil formation. From our results it is too early to comment on genetic relationships between soils, because variations in structure of organic matter between horizons of the same soil may be larger than differences between soils. Nevertheless, we can comment on humification processes. We have investigated the decomposition of plant litter by wet chemical and solid state NMR techniques. Detailed experimental data are available elsewhere 16. The CP-MASS-13C-NMR spectra of beech and pine leaves that have been microbiologically aged for 2 yr are shown in Fig. 4. Note that the major carbon types identified in some of the soils described in this study (Figs 1 and 2) are also those present in aged plant litter. The discrete

resonances in Figs 1 and 2 apparently represent decomposing organic matter, and the broad underlying resonances such as those in Fig. 3 represent humified material. Thus our results clearly show that CP-MASS-13C-NMR is ideal for studying the degree of humification and humification rates of soils.

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### Marshrock formed by iron sulphide and siderite cementation in saltmarsh sediments

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The formation of authigenic sulphides and other iron minerals has proved interesting both in studies of palaeomagnetism<sup>1</sup> and palaeosalinity reconstructions of Pleistocene and older sediments<sup>2,3</sup>. Iron sulphide and siderite-cemented nodules, concretions and layers are fairly common in the geological record<sup>4-6</sup>, but there is little evidence of their formation in modern environments. Accounts of authigenic iron minerals forming today in marine and brackish sediments generally refer only to 'micro-concretions' 10-500 µm in size, many of which have a 'framboidal' texture<sup>3,7,8</sup>. I describe here 'macroconcretions' and cemented layers which are now forming within intertidal marsh sediments at Warham, Norfolk, UK. Parts of these marshes are only 30 yr old, indicating how quickly early diagenetic cementation can occur. Siderite (FeCO<sub>3</sub>), greigite  $(Fe_3S_4)$  and possibly mackinawite  $(FeS_{1-x})$  are the main cementing agents. The term 'marshrock' is proposed for the cemented sediments by analogy with calcareous beachrock9,10. The formation of siderite-monosulphide concretions in the Warham sediments probably reflects a high 'reactive iron' content and limited sulphate arising from rapid sedimentation and burial of organic matter during the early stages of marsh

The Warham marshes form part of an almost continuous series which extend 35 km between Cley and Holme on the north Norfolk coast. They are open coast marshes, partly protected by barrier islands, spits and intertidal sand flats. Warham has an Upper Marsh (2.7-3.0 m OD) and a Lower Marsh (1.9-2.1 m OD), to seaward of which is an area of unvegetated intertidal sand flats (1.7-1.9 m OD)11. The Upper Marsh and Lower Marsh are covered by ~6% and 55% of tides respectively11

Cemented deposits have only been observed within the Lower Marsh sediments, and are best exposed along the banks of tidal creeks and a low cliff which marks its seaward edge, especially after spring tides when creek beds are subject to scour. Air photographs show that in 1948 most of the Lower Marsh area was unvegetated and possessed no distinct creek system, but that by 1960 vegetation was well established and the creek system

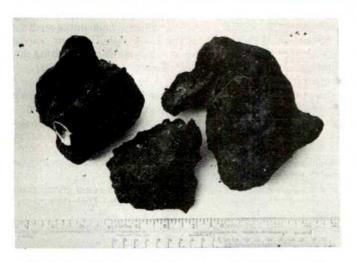


Fig. 1 Iron sulphide and siderite-cemented concretions from the Lower Marsh at Warham. Note the intact shell in the left-hand concretion.

had developed<sup>12</sup>. The marsh sediments are therefore mostly <30 yr old and the cemented layers and concretions must have formed within this time.

Observations suggest that cemented sediments occur discontinuously throughout the Lower Marsh area, but not in the Upper Marsh or intertidal flat sediments. The upper 1–1.5 m of Upper Marsh sediments are oxidized, but it is not known whether concretions and layers are present below this zone at elevations corresponding to those on the Lower Marsh. Preliminary observations elsewhere on this coast, however, support the conclusion that active concretion formation takes place only in younger lower marsh sediments.

The uppermost 30-40 cm of the Lower Marsh sediment column at Warham consists of brownish, organic-rich silty sands which overlie at least 1 m of black sands and silty sands. These colour zones correspond respectively to the 'hydroxide' and 'monosulphide' zones previously identified in Dutch coastal sediments<sup>13</sup> and in the Wash<sup>9</sup>. At Warham no lowermost grey 'bisulphide' zone is visible, but may be present beneath the monosulphide zone at depth.

The concretions and cemented layers are found within the black monosulphide zone, often in its upper part. Concretions are rounded and up to 40 cm in diameter (Fig. 1), but may be larger and more irregular in shape due to intergrowth of two or more neighbouring concretions. In some places continuous concretionary layers 15-25 cm thick can be traced laterally for 3 or 4 m. Many concretions and layers display 3-5 cm long tubular projections which often still contain recognizable rootlets. Some concretions also show clear horizontal grain size laminations which can be traced laterally into adjacent non-cemented sediments. Both these features testify that cementation has occurred within the marsh sediments and that the concretions are in situ. Some cemented material has been reworked in areas of localized bank erosion and is found as isolated fragments in creek beds and on the upper part of the intertidal flats. Such concretions usually have extensive surficial oxidation and bryzoan or algal encrustation.

Fresh specimens of cemented material are black though they may contain veins and patches of oxidized brown pigment. The concretions are hard, and there is a fairly sharp transition to the surrounding non-cemented sediment. Many specimens contain intact and broken shells, detrital chert pebbles, and fragments of macro-organic matter.

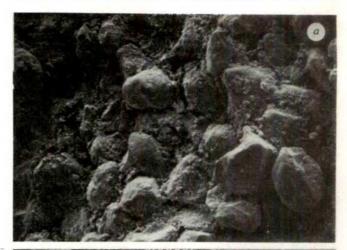
The material generally has a grain-supported fabric, with medium and fine-sized quartz representing >70% of the framework grains. Scanning electron microscopy (SEM) shows that the framework grains are cemented by rounded, granular aggregates or spherules  $2-5~\mu m$  in diameter (Fig. 2). The

spherules themselves seem to consist of individual particles up to 0.5 µm in size, many of which have an incomplete cubic form (Fig. 2b). Energy dispersive analysis (EDAX) under the SEM has detected large amounts only of iron and sulphur in these particles, with subsidiary aluminium, silicon and manganese.

The cemented sediments disintegrate completely within a few hours, with emission of H<sub>2</sub>S and CO<sub>2</sub>, when allowed to stand in cold HCl, suggesting that pyrite and marcasite, which are insoluble in HCl<sup>2,14</sup>, are not major components of the cement. Greigite, mackinawite and siderite are HCl-soluble, however. X-ray diffraction data (Table 1) confirm the presence of siderite and greigite, but do not confirm mackinawite or other form of FeS. The presence of a monosulphide other than greigite is suggested indirectly by the fact that some of the cement oxidizes rapidly to a brown colour on exposure to air, whereas greigite is stable in air and can withstand heating to 238 °C for 165 h without alteration<sup>15</sup>. Several unidentified X-ray peaks, which disappear after HCl treatment, may represent mackinawite or other monosulphide, although they do not correspond exactly to previous data for such minerals.

Siderite is apparently the major constituent of the cement in many concretions, although greigite and monosulphides are probably responsible for the black coloration. Approximately 20% of a sample of freshly crushed cement was attracted by a magnet, a property characteristic of greigite but not of siderite or mackinawite 15.

Both the formation of concretions and the dominance of siderite and greigite rather than pyrite in the Warham Lower Marsh may be explained in terms of the balance between 'reactive' iron and available sulphate (and therefore H<sub>2</sub>S) within the sediments. Berner<sup>16</sup> has demonstrated that both concretionary and layer concentrations of iron sulphide will probably



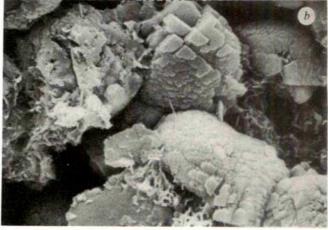


Fig. 2 Scanning electron micrographs showing: a, quartz framework grains and iron sulphide-siderite cement (picture width 1.3 mm); b, the cubic nature of some individual constituent particles of the iron sulphide spherules (picture width 100 μm).

Table 1 XRD data for two samples of marshrock cement (CuKα radiation)

а	b		
dÅ	dÅ		
5.52 s	5.42 vs		
5.37 s	5.32 s		Greigite?
3.82 vw	3.79 vw		
3.66 s	3.64 s	Siderite	
3.62 s	3.60 s		Greigite
3.22 vw	3.19 s		
3.02 s	2.99 m		Greigite
2.89 vw	2.85 w		
2.82 vvs	2.81 vvs	Siderite	
2.46 m	2.46 vw		Greigite
2.36 s	2.36 w	Siderite	
	2.25 w		
2.15 s	2.14 w	Siderite	
	2.09 vw		
1.98 s	1.97 w	Siderite	
1.86 w			Greigite
1.81 w	1.80 vw	Siderite	0.0.8.00
1.75 s	1.74 m	Siderite	Greigite
	1.67 vvw		
	1.59 vvw		
1.52 s	1.51 vw	Siderite	Greigite
	······		·····

vs, Very strong; s, strong; m, moderate; w, weak; vw, very weak.

form where there is a relatively high ratio of reactive iron to reducible sulphur. In such conditions outward diffusion of H<sub>2</sub>S from an organic-rich source area is restricted by immediate reaction with dissolved Fe2+ and resultant precipitation of FeS close to the source area. Because FeS is relatively insoluble, the concentration of dissolved iron in this area is reduced, thereby creating a diffusion gradient which allows further iron to move towards the incipient concretion.

The first-formed iron sulphide precipitates, mackinawite and greigite, are thermodynamically unstable relative to pyrite and should be converted to the latter by reaction with polysulphide ions 17-19. However, this diagenetic transformation can be arrested where sulphate availability is limited and monosulphides may be preserved<sup>20</sup>. This situation may occur in seawater but is most common in freshwater lakes and estuaries which contain less dissolved sulphate. Moreover, sediments which possess a high concentration of dissolved Fe<sup>2+</sup>, and in which the supply of H<sub>2</sub>S rapidly becomes depleted, may attain supersaturation with regard to non-sulphide iron minerals such as siderite and vivianite (Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>.8H<sub>2</sub>O), thereby leading to their precipitation<sup>21-24</sup>

In saltmarshes which do not occur within brackish water estuarine settings, as at Warham, the most important factor leading to rapid depletion of sulphate and H<sub>2</sub>S is probably a high rate of sediment accretion. For any given sulphate concentration, more rapid accretion results in more rapid bacterial sulphate reduction due to burial of more highly reactive organic matter<sup>25,26</sup>. If deposition is sufficiently rapid, the rate of sulphate reduction may be so great that the sulphate supply is exhausted before FeS is completely altered to pyrite<sup>1,26</sup>. On the north Norfolk coast, accretion rates on marshes less than 30 yr old are of the order of 1.0-1.7 cm yr<sup>-1</sup> (ref. 27), and may provide the conditions necessary for rapid sulphate exhaustion, monosulphide preservation and siderite formation. On older and higher marshes, accretion rates are much lower, of the order of  $0.002 \,\mathrm{cm}\,\mathrm{yr}^{-1}$  (ref. 27), and suitable conditions for these diagenetic processes are much less likely. This would explain why no concretions appear to be actively forming within the Upper Marsh at Warham and other 'high' marshes on the Norfolk coast. Because marsh accretion rates are initially most rapid and seem to decrease exponentially with time<sup>27</sup>, siderite/ greigite concretion formation may only occur in sediments deposited during the first few decades of marsh growth.

The nature of authigenic iron minerals formed in salt marsh sediments will depend on several factors other than accretion

rate (which reflects sediment availability and type, tidal regime and relative sea level movements), which include initial reactive iron content, the character of marsh vegetation (which controls organic production rates), and seasonal fluctuations in the activity of sulphate-reducing bacteria (which are largely temperature-controlled). Because these conditions vary between regions, marshes in different areas of similar age may possess distinctive suites of authigenic iron minerals. Sideritegreigite concretions and layers are unlikely to form in slowly accreting marsh sediments with a low reactive iron content; in such situations disseminated pyrite crystals and framboids would probably form very quickly, as has been reported from marsh peats in the eastern US<sup>28</sup>

The apparent importance of accretion rate as a control in the formation of authigenic iron minerals means that attempts to infer palaeosalinities associated with formation of iron concretions in fossil deposits must proceed with caution.

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## **Ipswichian fauna of Victoria Cave** and the marine palaeoclimatic record

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The lack of age dating methods which can be applied beyond the limit of radiocarbon dating makes the global correlation of continental climatic events and stratigraphic sequences with the continuous palaeoclimate record, obtained by isotopic and faunal analysis of deep-sea sediment cores 1-3, a difficult task; often only a relative time scale can be obtained using complex and perhaps tenuous litho- and biostratigraphical data. Speleothems may assist in this correlation, for embodied in their calcium carbonate structures are elemental and isotopic variations which indicate both their age and climatic conditions prevailing during their deposition. Speleothems may be reliably dated by the  $^{230}$ Th/  $^{234}$ U method to an age of  $\sim 350$  kyr BP providing they consist of non-porous, unrecrystallized calcite which is essentially free of clastic detrital sediments4-6. They

may also provide palaeoclimatic information for previously glaciated areas (1) because their presence indicates that groundwater movement was not prevented by ice formation at the surface, and CO2 was produced in the soil zone by root respiration and plant decay (limestone bedrock may then be dissolved and reprecipitated as speleothems in caves below the surface), and (2) because of the variations in stable isotopic content and fluid inclusion waters contained therein4.7.8. Additional climatic and chronological information may be obtained where speleothems are interstratified with deposits characteristic of a particular climatic regime, and with deposits containing organic remains. We describe here the results of dating speleothems encrusting the remains of mammals from Victoria Cave in northern England, and discuss their significance in terms of the correlation between continental and oceanic palaeoclimatic records.

· SEEDER SEEDER

Victoria Cave is situated at an altitude of 440 m a.s.l. in a Carboniferous limestone scar north-east of Settle, North Yorkshire. The Victoria Cave Exploration Committee of the British Association for the Advancement of Science conducted excavations at the site from 1870 to 1878 and published details of the Pleistocene deposits9. Two quite distinct fossiliferous horizons were found, but we will consider here only the older of these, the Lower Cave Earth. Tiddeman records an interglacial mammal fauna from this deposit which included such animals as hyaena, hippopotamus and narrow-nosed rhinoceros. He observed that the Lower Cave Earth was truncated at the cave mouth by glacial till, while inside the cave it was overlain by laminated clays. It was reasoned that the till and laminated clays were laid down during the last glacial period and that the lower Cave Earth and its contained fauna belonged to the preceding interglacial. The relationship of these deposits is shown in Fig. 1.

Further (unpublished) excavations by Lord in 1937 revealed mammal bones, teeth and deer antler lying on a surface within the Lower Cave Earth, covered by a layer of calcite flowstone to a depth of  $\sim 10$  cm. Their unweathered condition indicated that the time between their introduction into the cave and their burial beneath the flowstone was not great. All the samples from which the present series of  $^{230}$ Th/ $^{234}$ U dates are derived were taken from blocks found during Lord's excavations, now housed in the Pigyard Museum at Settle. Re-examination of material from Victoria Cave has confirmed the presence of the above mammals recorded by Tiddeman in the Lower Cave Earth (and its interbedded speleothems). A list of the Victoria fauna is given in Table 1.

This faunal assemblage is a classic example of the late Pleistocene 'hippopotamus fauna' as defined by Sutcliffe<sup>10</sup> on the basis of material from Joint Mitnor Cave in Devon. He assigned the assemblage to the warmest part of the Eemian (=Ipswichian) interglacial. Palaeobotanical evidence from Trafalgar Square, London<sup>11</sup> and Barrington, Cambridgeshire<sup>12</sup> has demonstrated the association of the 'hippopotamus fauna' with

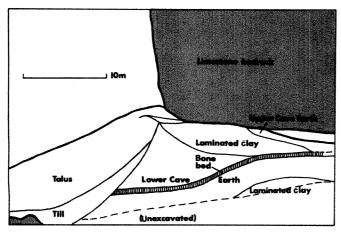


Fig. 1 Diagrammatic longitudinal section of the Pleistocene deposits of Victoria Cave.

Table 1 Fauna of the Lower Cave Earth in Victoria Cave

Species identified	Common name
Ursus arctos	Brown bear
Crocuta crocuta	Spotted hyaena
Panthera leo	Lion
Palaeoloxodon antiquus	Straight-tusked elephant
Dicerorhinus hemitoechus*	Narrow-nosed rhinoceros
Hippopotamus amphibius*	Hippopotamus
Megaloceros giganteus*	Giant deer
Cervus elaphus*	Red deer
Bos or Bison sp.	A bovine

<sup>\*</sup> Species present in the dated flowstone samples.

interglacial pollen spectra representing a period of climatic optimum (zone Ip IIb). At Swanton Morley, Norfolk, there is evidence for the continued existence of *Hippopotamus* into the declining phase of the interglacial (zone Ip III)<sup>13</sup>.

The 'hippopotamus fauna', which is represented at many localities in England and Wales, is remarkably constant in its composition and there is no evidence to suggest that this particular species grouping was present more than once in the British sequence. Many fluviatile deposits in southern Britain have produced an extraordinary abundance of remains of hippopotamus, yet it is virtually absent from similar deposits in adjacent parts of the continent strongly suggesting that we are dealing with an island fauna, and therefore, a period in which sea-level was sufficiently high to isolate Britain from Europe. Rare occurrences of *Hippopotamus* in France, in apparent 'Riss-Würm' contexts, may chart this animal's route from the Mediterranean area.

At Sewerby Cliff, Holderness, elements of the 'hippopotamus fauna' have been found in beach deposits in front of a buried cliff feature at 2-3 m above OD, slightly higher than the level of modern high water during spring tides at the same locality <sup>14</sup>. The elevation of the deposits suggests a correlation with the high sea-stand of isotope substage 5e <sup>1</sup>, thus precluding a younger age for the 'hippopotamus fauna'. However, their elevation may be due, in part, to local isostatic movement and crustal adjustment, so that correlation with the younger substages, 5c and 5a, cannot be completely ruled out unless the deposits can be assigned an absolute age. This has been possible in the case of the Victoria Cave fauna by dating the speleothems associated with the bone remains.

Seven samples of flowstone were analysed by the  $^{230}$ Th/ $^{234}$ U dating method, using analytical techniques and age calculations described elsewhere  $^{15}$ . The accuracy and precision of ages determined by this method are demonstrated by: (1) 12 analyses of a homogenized speleothem standard (mean age 47.8 kyr BP) have shown that the mean error determined by averaging  $1\sigma$  counting uncertainties ( $\pm$ 2.2 kyr) is comparable to the standard deviation of the determined ages ( $\pm$ 1.7 kyr) $^{16}$ ; (2) close agreement of ages determined by the McMaster laboratory with other laboratories for several homogenized carbonate standards has been found in the uranium-series interlaboratory comparison project  $^{17}$ , and (3) results of duplicated analyses of the same sample of several U-rich speleothems using the  $^{231}$ Pa/ $^{230}$ Th dating method show good agreement with  $^{230}$ Th/ $^{234}$ U ages  $^{16}$ .

All samples analysed consist of yellow to brown laminated calcite enclosing or overlying bones. Sections cut through the flowstones clearly showed that the calcite post-dated the bone. The results of 12 analyses are shown in Table 2. Except for three results (79000-1, 79001-1 and 79025-1) all ages fall within the interval 135-114 kyr BP. Re-determination of these three apparently younger samples gave ages within the 135-114 kyr interval (79000-2, 79001-3, -4, 79025-3). The initial younger ages are thought to be due to slight contamination by uranium which had migrated from the relatively U-rich fossil bones into the immediately adjacent calcite. The replicates were taken from calcite more removed from (and therefore stratigraphically younger than) the initial analyses, and hence were less likely to

Table 2 Sample descriptions, isotopic ratios and <sup>230</sup>Th/<sup>234</sup>U ages for seven samples of flowstone containing mammal remains from Victoria Cave, northern England

Speleothe	m		Analysis	U	The second secon			A
no.	Description	No.	Location	(p.p.m.)	$^{234}U/^{238}U$	$^{230}Th/^{232}Th$	$^{230}Th/^{234}U$	Age (kyr)
79000	Brown laminated flowstone	-1	Adjacent to teeth	0.62	$1.000 \pm 0.067$	44	$0.610 \pm 0.041$	102+12*
	enclosing teeth of	-2	~1 cm away from teeth	0.63	$1.019 \pm 0.021$	20	$0.672 \pm 0.021$	$120\pm7$
	Dicerorhinus hemitoechus		·				0.00	12027
79001	Yellow-brown flowstone	-1	Adjacent to bone	0.50	$1.100 \pm 0.028$	20	$0.623 \pm 0.022$	$104 \pm 6$
	enclosing jaw of	-2	~2 cm away from bone	0.40	$1.033 \pm 0.031$	152	$0.691 \pm 0.027$	$126^{+10}_{-9}$
	Dicerorhinus hemitoechus	-3	~2 cm away from bone	0.43	$1.022 \pm 0.029$	34	$0.714 \pm 0.022$	135±8
79002	Calcite enclosing antler	-1	Adjacent to bone	0.50	$1.012 \pm 0.026$	16	$0.702 \pm 0.024$	131 ± 8
	of Cervus elaphus		•				01102 - 01027	151-8
79021	Flowstone enclosing teeth	-1	2 cm adjacent to teeth	0.43	$1.057 \pm 0.024$	31	$0.690 \pm 0.019$	$125^{+7}_{-6}$
	of Dicerorhinus hemitoechus		•			31	0.070 2 0.017	1206
79023	Flowstone and pool calcite	-1	1 cm of clean side of	0.50	$0.994 \pm 0.022$	71	$0.678 \pm 0.020$	$123 \pm 7$
	enclosing teeth of		flowstone			, ,	0.070 - 0.020	12511
	Megaloceros							
79025	9-cm deep section of flowstone	-1	0.5 cm above base	0.88	$1.120 \pm 0.021$	111	$0.484 \pm 0.014$	$71 \pm 3$
	block which contains large		(at bone level)			***	0.404 - 0.014	71113
	mammal bones at its base	-2	Top 1.5 cm	0.43	$1.048 \pm 0.019$	84	$0.654 \pm 0.016$	$114 \pm 5$
	including calcaneum of		0.5 to 1.5 cm above base	0.58	$1.037 \pm 0.018$	47	$0.672 \pm 0.019$	$120 \pm 6$
	Hippopotamus	-		5.50	2.00 0.010	7,	0.01220.013	12020
79026	Flowstone enclosing teeth of	-1	Top (?) 2 cm of	0.46	$1.022 \pm 0.016$	>1,000	$0.668 \pm 0.016$	119±5
	Dicerorhinus hemitoechus	•	flowstone	0.40	1.022 - 0.010	~ 1,000	0.000 ± 0.010	11923

<sup>\*</sup> U yield = 6%. All other yields are >20%. All error limits are  $\pm 1\sigma$ .

be influenced by nuclide migration from the bone. The appreciably higher U content of the most anomalous age determination (79025-1) further supports this interpretation, although this distinction is not seen in the case of 79000.

The results indicate that members of the 'hippopotamus fauna' were present in the vicinity of Victoria Cave during or before the interval  $135 \pm 8$  to  $114 \pm 5$  kyr BP. More specifically, because the basal and top ages of sample 79025 are in normal stratigraphic sequence, and because the bone layer lies at the base of this deposit, the fauna of the Lower Cave Earth can be placed at or before 120 ± 6 kyr BP. This compares very favourably with the date of 125-120 kyr BP for substage 5e of the marine isotopic record<sup>1,18</sup>.

The association of Ipswichian pollen assemblages with the 'hippopotamus fauna' at several sites would suggest that the Ipswichian interglacial sensu stricto must therefore also coincide with substage 5e. However, the paucity of vertebrate fauna at the Ipswichian type locality at Bobbitshole, near Ipswich, Suffolk, makes a direct correlation with the Lower Cave Earth in Victoria Cave impossible. Elsewhere, there are reported discrepancies between what are apparently Ipswichian pollen assemblages and the associated mammal faunas, suggesting that some of the pollen assemblages assigned to the Ipswichian may actually represent other warmer stages<sup>19,20</sup>. Many of these difficulties arise from the unsuitability of the chosen type locality for this important phase of the Pleistocene. Despite such problems, the term 'Ipswichian' is widely applied to the last period in which the climate of the British Isles was as warm as, or warmer than, at present, though this is not strictly within the original definition of the term. This procedure is necessitated by the absence of any other acceptable name for the same phase. With this qualification, we feel justified in correlating the 'Ipswichian interglacial' of the British terrestrial sequence with substage 5e of the marine isotope record.

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## Phosphotyrosine-containing proteins isolated by affinity chromatography with antibodies to a synthetic hapten

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Tyrosine-specific protein kinases seem to be involved critically in cellular transformation by tumour viruses1-4, and may also be involved in the cellular response to epidermal growth factor<sup>5</sup>. To facilitate the identification and isolation of phosphotyrosinecontaining proteins (PT-proteins) we have sought to develop as a general reagent anti-O-phosphotyrosine antibodies. We show here that affinity chromatography with these antibodies is effective for the small-scale isolation of some PT-proteins, including p120, the transforming protein of Abelson murine leukaemia virus, and several unidentified proteins from Rous sarcoma virus (RSV)-transformed mouse fibroblasts.

New Zealand rabbits were injected repeatedly intradermally (i.d.), each time with ~1 mg of keyhole limpet haemocyanin containing ~30 p-azobenzyl phosphonate<sup>6</sup> (ABP) groups per 100,000 molecular weight  $(M_r)$ . Benzyl phosphonate, rather than phenyl phosphate, was chosen to avoid the loss of haptenic groups by the action of tissue phosphatases. For the first injection, the antigen was incorporated in complete Freund's adjuvant; for subsequent ones (on days 23, 33, 40 and 92) it was given in incomplete Freund's adjuvant. Anti-ABP activity in serum was monitored by a solid phase radioimmunoassay in which small tubes (Dynatech) coated with ABP<sub>30</sub>-bovine serum

albumin (BSA) were incubated with the rabbit antisera, washed and finally tested with <sup>125</sup>I-labelled *Staphylococcus aureus* Protein A to measure the extent of binding of rabbit immunoglobulin to the ABP-BSA.

Antibodies were isolated from the active sera by affinity chromatography on Sepharose to which was attached O-phosphotyramine. The latter was prepared by phosphorylating tyramine with P<sub>2</sub>O<sub>5</sub> (ref. 7). A strongly coloured side-product was precipitated by the addition of 1 vol ethanol and 13 vol n-butanol. O-phosphotyramine was then precipitated by addition of 15 vol ethyl acetate and recrystallized twice from ethanol/water. The recrystallized product migrated as a single component on TLC using isopropanol/H2O/conc. NH4OH (7:2:1) as solvent. On silica gel  $(R_F = 0.1)$  it was detected using  $I_2$ , ninhydrin and by UV absorption, and on cellulose ( $R_F = 0.2$ ) it was detected with ninhydrin. Its NMR spectrum was consistent with the expected structure. The recrystallized O-phosphotyramine was coupled to cyanogen bromide (CNBr)activated Sepharose 4B (30 mg per g) in 0.1 M NaHCO<sub>3</sub>. Immunoglobulins from anti-ABP serum (precipitated with 40%

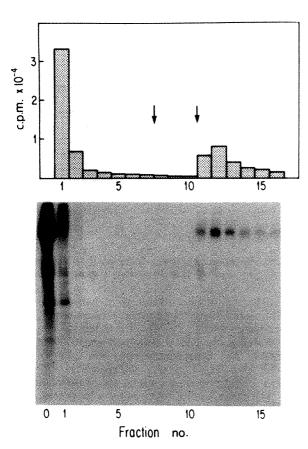


Fig. 1 Affinity chromatography of phosphotyrosine-containing proteins from an extract of Abelson virus-transformed B lymphocytes (ANN-1 cells). A crude preparation of p120 (40 μg) was phosphorylated by reaction for 30 min on ice with 5 μCi[γ-³2P]ATP in 10 mM MnCl<sub>2</sub>, 1 mM phenylmethylsulphonyl fluoride (PMSF), 100 kallikrein units aprotinin ml<sup>-1</sup>, 150 mM Tris pH 8.0. After addition of 20 mM Na<sub>2</sub>-EDTA, the reaction mixture was diluted with an equal volume of buffer I (0.5% NP-40, 150 mM NaCl, 100 units aprotinin ml<sup>-1</sup>, 0.1 mg ml<sup>-1</sup> ovalbumin, 10 mm Tris pH 8.0) and applied to a 0.15 ml Sepharose column of anti-ABP antibodies (0.75 mg immunoglobulin per ml Sepharose). The column was washed with buffer I (fractions 2-7), then with buffer II (fractions 8-10) and finally with buffer III (fractions 10-16). Buffer II differed from I only in that II contained 20 mM phosphoserine, 20 mM phosphothreonine, 50 mM NaCl and 3.3 mM Na<sub>2</sub>HPO<sub>4</sub>. Buffer III differed from II in that III had 40 mM phenyl phosphate in place of phosphoserine and phosphothreonine. Each fraction was 0.5 ml. The protein precipitated with 5% perchloric acid from a 25 μl sample of each fraction was collected on a Millipore filter and counted in Bray's solution (upper panel). Of the total perchloric acid-precipitable radioactivity applied to the column, ~50% was recovered. A sample of each fraction and of the original reaction mixture (fraction 0') was also analysed by electrophoresis in 10% SDS-polyacrylamide gels<sup>13</sup>, followed by autoradiography of the dried gels (lower panel). The 'fraction 0' sample had half the volume of the other fractions.

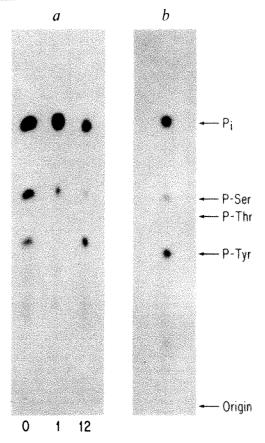


Fig. 2 Phosphoamino acid analyses by high voltage paper electrophoresis. Proteins were precipitated with 5% perchloric acid and washed successively with 5% perchloric acid, 5% trichloroacetic acid and acetone. The samples were hydrolysed for 2 h at 100 °C in 6M HCl, dried and subjected to high voltage electrophoresis in pyridine-acetic acid (pH 3.5) on Whatman 3-MM paper at 2,400 V for 1 h. a, Samples from the reaction mixture (fraction 0), and fractions 1 and 12 of the column described in Fig. 1. b, Phenyl phosphate-eluted material from RSV-transformed fibroblasts labelled as intact cells with <sup>32</sup>P-inorganic phosphate (see Fig. 3 legend), P-Ser, phosphoserine; P-Thr, phosphothreonine; P-Tyr, phosphotyrosine.

saturated ammonium sulphate) were applied to the phosphotyramine-Sepharose column at room temperature. After washing the column with buffer (150 mM NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub> pH 7.4) at room temperature and then at 45 °C, the retained antibodies were eluted at 45 °C with modified buffer containing 40 mM phenyl phosphate, 50 mM NaCl and 3.3 mM NaH<sub>2</sub>PO<sub>4</sub> pH 7.4. Phenyl phosphate was removed from the eluate by dialysis. The hapten-eluted protein accounted for

Table 1 Specificity of anti-ABP antibodies measured by competition radioimmunoassay

Competitor	% Inhibition
Phosphotyrosine (25 µM)	2
Phosphotyrosine (74 µM)	8
Phosphotyrosine (220 µM)	16
Phosphotyrosine (670 µM)	28
Phosphotyrosine (2 mM)	48
Phosphoserine (2 mM)	<1
Phosphothreonine (2 mM)	< 1

Anti-ABP antibodies (3.3  $\mu$ g in 50  $\mu$ l phosphate-buffered saline (PBS)) were incubated in polystyrene tubes (Dynatech) for 1 h at room temperature. After washing, the tubes were incubated for 1 h at room temperature with 9 ng  $^{125}$ I-(azophenyl phosphate)<sub>7</sub>-RNase and the above inhibitors in 20  $\mu$ l PBS. The tubes were then washed and counted. In the absence of inhibitors  $\sim 30\%$  of  $^{125}$ I-labelled probe was bound. The high concentration of phosphotyrosine required for inhibition was probably due to the multivalency of the azophenyl phosphate-RNase.

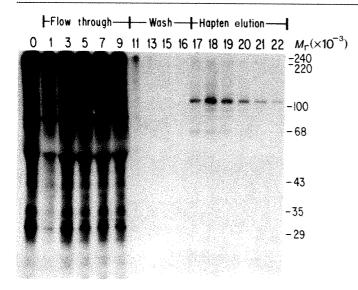


Fig. 3 Isolation of phosphotyrosine-containing proteins from RSV (Schmidt-Ruppin strain)-transformed mouse (BALB/c) fibroblasts;  $3\times10^6$  cells were incubated with 0.25 mCi  $^{32}PO_4$  in phosphate-free medium for  $3\frac{1}{2}$  h. The cells were washed with ice-cold medium and extracted three times with 1 ml ice-cold 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 0.1% BSA, 10 mM Na<sub>2</sub>-EDTA, 10 mM NaF, 150 mM NaCl, 100 units aprotinin ml<sup>-1</sup>, 1 mM PMSF, 10 mM sodium phosphate pH 7.3. After scraping residual material from the plastic surface with a rubber policeman the extract was homogenized (twelve strokes in a Dounce homogenizer) and centrifuged at 105,000g for 1 h. The supernatant was generally stored at -20 °C after addition of glycerol to 30% (v/v). After thawing, samples were mixed with 1 vol buffer I (see Fig. 1) and applied to the column (fractions 1-5), followed by additional buffer I (fractions 10-16) and finally by elution with buffer III (fractions 17-22), which contained phenyl phosphate. The protein from 90 µl of each 0.5 ml fraction was precipitated with 1 ml acetone (-20 °C), boiled for 2 min in SDS-2-mercaptoethanol and subjected to SDS-polyacrylamide gel electrophoresis. This figure shows the autoradiograph of the dried gel. Sample 0 is the 105,000g supernatant of the total cell extract. Molecular weight markers are indicated at the right. The faint band at  $M_r = 36,000$  might correspond to the <sup>32</sup>P-p36 protein described elsewhere <sup>14,15</sup>.

~95% of the ABP-binding activity of the immunoglobulin fraction and corresponded to 0.9 mg antibody per ml serum. By fluorescence quenching<sup>8</sup> the affinity (equilibrium association constant) of the purified antibodies for the 2,4-dinitrophenyl (DNP) derivatives of p-aminobenzyl phosphonate and of paminophenyl phosphate was estimated to be  $\sim 4 \times 10^6 \, \text{mol}^{-1}$  at 20 °C. (Because of the simplicity of these ligands and the DNP moiety which was introduced to facilitate affinity measurement<sup>10</sup>, the observed equilibrium constants are only approximations of the affinity for phosphotyrosine in PT-proteins.) These ligands did not specifically quench the fluorescence of anti-phenyl arsenate antibodies. As judged by competitive radioimmunoassay with 125I-(azophenyl phosphate)7-ribonuclease as the radiolabelled ligand, the anti-ABP antibodies had no measurable affinity for O-phosphoserine or O-phosphothreonine (Table 1). (The azophenyl phosphate-ribonuclease was prepared from diazotized p-aminophenyl phosphate, which was synthesized as described above for O-phosphotyramine.)

An 'affinity' column, prepared by coupling the purified anti-ABP antibody to CNBr-activated Sepharose, was tested for its ability to bind a crude preparation of the transforming protein (p120) of Abelson murine leukaemia virus. This protein (given by A. Dasgupta) was phosphorylated with  $[\gamma^{-32}P]$ ATP in conditions where essentially all the  $^{32}P$  incorporated into p120 was in the form of O-phosphotyrosine<sup>2</sup>. The <sup>32</sup>P-p120 was retained by the column, from which it was specifically eluted by phenyl phosphate, but not by phosphoserine or phosphothreonine (Fig. 1). In other experiments (not shown), neither 40 mM p-nitrobenzoic acid nor 10 mM sodium phosphate eluted the p120. Moreover, phosvitin, which is rich in phosphoserine and phos-

phothreonine but lacks O-phosphotyrosine, did not bind to the affinity column. The failure of the phosphorylated p120 to adhere to a similar Sepharose column that had been prepared with anti-phenyl arsenate antibodies in place of the anti-benzyl phosphonate antibodies provided additional evidence of specificity. (Antibodies to phenyl arsenate have been reported to cross-react strongly with phenyl phosphonate11; because these antibodies did not retain PT-proteins they probably react only weakly with benzyl phosphonate and phenyl phosphate.)

Aliquots of the column fractions described in Fig. 1 were hydrolysed with 6 M HCl and analysed by high voltage paper electrophoresis (Fig. 2a). O-phosphotyrosine was virtually absent from hydrolysates of the pass-through from the affinity column and was enriched in the <sup>32</sup>P-protein eluted by phenyl phosphate.

The affinity column was also able to bind <sup>32</sup>P-PT-proteins that had been formed by incubating intact cells with <sup>12</sup>P-labelled inorganic phosphate (Pi). Schmidt-Ruppin (RSV)-transformed mouse fibroblasts labelled in this way, were lysed by detergent and the lysates applied to the affinity column. Figure 3 shows an SDS-polyacrylamide gel electrophoretic analysis of the proteins that were applied to and eluted from the column, and Fig. 2b shows the <sup>32</sup>P-phosphoamino acids in hydrolysates of the eluted proteins. It is evident that the affinity column selected a discrete set of phosphoproteins highly enriched in O-phosphotyrosine. One of the conspicuous PT-proteins detected (PT-110) has not to our knowledge been previously reported, perhaps because of its high molecular weight ( $M_r = 110,000$ ; Fig. 3)<sup>12</sup>. Because the amount of PT-110 isolated from the RSV-transformed cells was much greater than that obtained from uninfected cells (Y. Onodera, personal communication), PT-110 may be a substrate for pp60src, or for another kinase activated by pp60src, and may play a part in expression of the transformed phenotype.

The specific retention of PT-proteins by antibodies to benzyl phosphonate depends on cross-reactions in which the antibodies have sufficiently high affinity for O-phosphotyrosine and negligible affinity for O-phosphoserine and O-phosphothreonine. The intrinsic affinity for O-phosphotyrosine has to be relatively high because there is probably only a single O-phosphotyrosine residue per PT-protein and, therefore, few if any opportunities for multivalent binding. It is likely that the anti-benzyl phosphonate antibodies do not have the same affinity for every PT-protein because of local differences in charge density and in accessibility of phosphotyrosine residues. Nonetheless, it seems from preliminary data that ~50-60% of the <sup>32</sup>P-PT-proteins were recovered in the phenyl phosphate eluate. Phenyl phosphate, rather than denaturing agents, was used to recover PT-proteins from the column so as to enhance the specificity of the elution and to prolong the useful life of the column. It is possible that higher affinity antibodies can be generated using haptenic groups, for example, p-azophenyl phosphate, that are closer in structure to phosphotyrosine than p-azobenzyl phosphonate.

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# Hot spots of frameshift mutations induced by the ultimate carcinogen N-acetoxy-N-2-acetylaminofluorene

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An important step in carcinogenesis is thought to be the initial attack of the DNA molecule by a so-called ultimate carcinogen. More than 90% of the carcinogens tested have been found to be mutagens in bacterial systems1. The covalent binding of the ultimate carcinogen to the DNA bases or phosphate groups creates a premutational lesion that in vivo is processed by the repair, replication and recombination enzymes, and eventually may be converted into a mutation. Being interested in the way that an initial premutational event is converted into a stable heritable mutation, we have sequenced stable mutations in a gene that has formed covalent adducts in vitro with N-acetoxy-N-2-acetylaminofluorene (N-AcO-AAF, a model for the ultimate metabolite of the rat liver carcinogen 2-acetylaminofluorene AAF). We show here that the mutations are mainly frameshifts involving G · C base pairs, and that certain base pairs (hotspots) are affected at relatively high frequences. These results fit a model in which N-AcO-AAF-modified guanine acts as a non-coding base that during replication results in deletion of the modified residue.

In vivo studies have shown the mutagenicity of AAF and its derivatives in both bacterial<sup>2,3</sup> and eukaryotic<sup>4</sup> systems. N-AcO-AAF reacts in vitro with DNA leading mainly to the formation of a guanine adduct<sup>5</sup>, N-2-(deoxyguanosin-8-yl)-acetylaminofluorene (80%) and to at least three minor adducts (N.S., R.P.P.F. and M.P.D., unpublished results), one of which has been characterized as 3-(deoxyguanosin-N<sup>2</sup>-yl)-acetylaminofluorene<sup>6</sup>. Studies by our group showed that binding of N-AcO-AAF to DNA resulted in a local distortion of the DNA helix around the C-8 adduct<sup>7-9</sup>, which we have called the insertion-denaturation model<sup>10</sup>. A similar model has been proposed by other investigators<sup>11</sup>. We describe here the analysis

of forward mutations induced in the tetracycline-resistance gene of pBR322 by directing the chemical reaction of the carcinogen to a small restriction fragment (BamHI-SalI) inside the antibiotic-resistance gene. These two restriction enzymes each cut pBR322 only once and both sites reside in the tetracyclineresistance gene. The strategy used is outlined in Fig. 1. The resulting small restriction fragment (275 base pairs (bp); 6S fragment) modified to various extents with N-AcO-AAF (3H ring) was reinserted by in vitro ligation into the non-reacted large (BamHI-SalI) pBR322 restriction fragment (16S fragment). The ligation mixture was used to transform CaCl2treated Escherichia coli recipient cells. Mutants are selected for ampicillin (Ap) resistance and tetracycline (Tc) sensitivity. The induction of SOS functions by UV irradiation of the cells before transformation increased the observed mutation frequency. The plasmid DNA of such mutants was analysed for sequence changes in the fragment where the AAF binding had been directed.

Mutation frequencies were calculated as the ratio of ApR Tc<sup>S</sup>/Ap<sup>R</sup> clones. The frequency in the control experiment in which the 16S fragment was ligated to a non-modified 6S fragment was 0.4% when the bacteria were not treated with UV before the transformation step; when UV treatment was applied, the frequency was 0.6%. When analysed by gel electrophoresis, the plasmid DNAs isolated from such clones were always shorter than the original pBR322, in general by 0.2-0.8 kilobases (kb). The restriction analysis pattern showed that these mutant DNAs had retained the unique EcoRI site but that in general they had lost both the BamHI and SalI restriction sites. We call these mutants class I mutants and suggest that they mainly arise from the dimerization of the 16S fragment. Such dimers, which have the Ap<sup>R</sup> Tc<sup>S</sup> phenotype, are then converted to smaller plasmids (monomers) through *in vivo* recombination. Work is in progress to lower this mutation background by using an alkaline phosphatase-treated 16S fragment<sup>12</sup>. Class I mutants were easily recognized and excluded from the pool of mutants to be sequenced.

When 6S-AAF fragments ligated to the non-modified 16S fragment are used to transform *E. coli* the transformation efficiency decreases with increasing levels of bound AAF residues (Fig. 2). The extent of this AAF-dependent inactivation of transformation is strongly related to the general repair genotype of the recipient cell (R.P.P.F. and E. Seeberg, in preparation). One also finds a corresponding increase in the mutation

Table 1 Properties	of variou	s mutants
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Description of mutation	Mutant no.	Strain	Induction of SOS functions	Average no. of bound residues per 6S fragment	Sequence near the n	autation
	4	AB 1886	+	2.0	511 524	529
-1 deletion of G 520 or 521	30	AB 1886	-	2.8	GGGTATGGTGGC	AGGCCCG
	41	AB 1157	+	13.8		
	45	AB 1157	+	2.8		
					381 390	399
-1 deletion of G 389 or 390	32	AB 1157	+	8.8	TCTACGCC[G]AC	GCATCGT
					428	446
-2 deletion of GC 435-436	34	AB 1157	+	6.6	GTTGCTGGCGCC	TATATCG
or 437–438 or CG 436–437	36	AB 1157	+	8.8		
Addition of a C within	36				518 +1 <u>C</u>	536
sequence 526–528	50				GTGGCAGGCCCG	TGGCCGG
-2 deletion of GC 548-549					540	558
or 550–551 or CG 549–550	33	AB 1157	+	13.8	ACTGTTGGGCGC	CATCTCC
-1 deletion of G 416 and					406	424
double transition at 414–415	35	AB 1157	+	8.8	$\begin{array}{c} CATCACCG[\overline{GCG}]C\\ double & \uparrow -1\\ transition & \overline{AT} \end{array}$	CACAGGT

The sequences in the table are the wild-type sequences with the numbering defined by Sutcliffe<sup>15</sup>. The bases involved in the deletion mutations are boxed with dotted lines. The different possibilities to obtain a given mutated sequence are shown. Mutant 36 exhibits two mutations: a-2 deletion as in mutant 34 and a+1 addition of a C residue within the sequence CCC at positions 526-528. Mutant 35 has got a-1 deletion of a G at position 416 and a double transition,  $GC \rightarrow AT$ , at position 414-415.

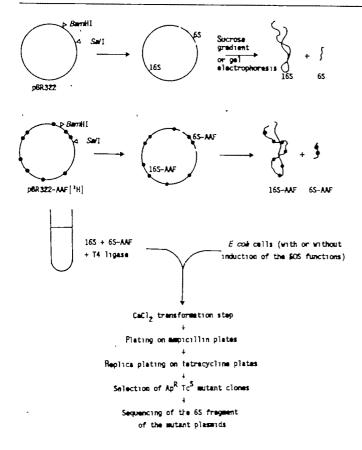


Fig. 1 Strategy for the site-directed mutagenesis experiments. The  $E \, coh$ strains used were AB 1157 or AB 1886 (ref. 18), N-AcO-AAF (<sup>9</sup>H ring) was synthesized as described previously<sup>10</sup> (specific activity 196 mC mmol<sup>-1</sup>) N-AcO-AAF ('H ring) reaction with supercolled plasmid DNA was performed in 10 mM ris, 1 mM EDTA, pH 8 (TE) buffer containing 5% of ethanol (DNA concentration 50 µg ml<sup>-1</sup>). Removal of unbound fluorene derivatives was achieved by four successive ethanol precipitations. The number of AAF residues bound per plasmid molecule was determined as previously described20. Samples of pBR322 reacted with N-AcO-AAF to various extents (ranging from 0 to 2.5% of modified bases) were digested with Bamili and Sali restriction emymes (Boehringer, Mannheim). The large (16S) and the small (6S) fragments were separated and purified either by velocity sedimentation on sucrose gradients (5-20%) or by electro-phoresis on 0.8% agarose or on 8% polyacrylamide gels followed by electroelution T4 DNA ligase (Biolabs) was used to ligate the unmodified 16S fragment with either the unmodified 6S fragment or the 6S fragments obtained from the various AAF-modified pBR322 samples (6S-AAF). The ligation was performed in the conditions specified by the T4 DNA ligate manufacturer. The DNA fragment concentrations were  $16.5 \, \mu g \, \mathrm{ml}^{-1}$  for the  $168 \, \mathrm{fragment}$  and  $7.5 \, \mu g \, \mathrm{ml}^{-1}$  for the  $68 \, \mathrm{fragment}$ . UV irradiation of the cells before transformation. In some cases, the E coli cells were UV irradiated before the transformation procedure to induce the cellular SOS response The cells were UV irradiated as a suspension in 0.01 M MgSO. with a germicidal lamp (15 W, Phillips) at a dose giving about 50% survival (that is, 60 J m<sup>-2</sup> for the wild-type strain, AB 1157, 6 J m<sup>-2</sup> for the wind strain, AB 1886) The cells were then incubated in LB medium for 30 mm at 37 °C to allow expression of the SOS functions. E. coli transformation and selection of the ampicillim-resistant (Ap<sup>®</sup>) and tetracycline-sensitive (Tc<sup>®</sup>) clones. The E coll were made competent for transformation by the classical  $CaCl_2$  treatment procedure<sup>21</sup>. The different ligation mixtures were diluted by a factor of 100 in (10 mM Tris), 10 mM CaCl<sub>2</sub> and 10 mM MgCl<sub>2</sub> (pH 7) and used to transform the competent cell suspension by mixing 1 vol of the DNA solution with 2 vol of the concentrated B coli suspension. Following the transformation procedure, the cells were spread on LB plates containing ampicilin (50 µg ml<sup>-1</sup>) and incubated at 37 °C overnight. The closes were then replated on LB plates containing tetracycline (20 µg ml<sup>-1</sup>). Clones growing on Ap but not Tc were scored as Ap<sup>3</sup>, Tc<sup>3</sup> mutants. Such individual mutant clones were then grown further in LB medium plus ampicillin for preparation of the plasmid DNA contained in these clones. Plasmid DNA was purified either on a small scale (10 ml of culture) by an adaptation of the method of Clewell and Heimski<sup>23</sup> or on a larger scale (11 culture) by a NaCl/SDS lysas procedure followed by a CsCl/ethadrum bromude centrifugation step <sup>23</sup>. DNA sequence analysis of the mutants. Class II mutant plasmids (see text for the definition) were digested with BamHI and SaII restriction enzymes and <sup>32</sup>P-end-labelled at their 5' extremnties with T4 DNA kinase (Bochringer, Mannheim). Strand separation and sequencing were performed according to the method of Maxam and Gilbert<sup>14</sup>.

frequency, provided the cells are exposed to UV before the transformation step. The mutant DNAs isolated from such experiments fall into two classes when analysed by gel electrophoresis: class I mutants defined as in the control experiment, and class II mutants, exhibiting the original size of pBR322 and retaining both BamHI and SalI restriction sites.

The frequency of class II mutants increases with the level of AAF modification and reaches about 3% at the highest level tested (Fig. 2). Note that only the class II mutation frequency is (1) a function of AAF modification and (2) dependent on UV irradiation of the host cell (for the conditions, see Fig. 1 legend). Both these properties indicate that class II mutants, as opposed to class I mutants, are actually induced by AAF adducts and that they require efficient expression of the cellular SOS functions. We, however, also obtained class II mutants, but at a lower frequency, in conditions where the SOS functions were not induced with UV light, tending to support the hypothesis that there is a constitutive level of error-prone repair<sup>13</sup>.

Class II mutants can also theoretically arise from 'non-targeted' mutations involving the SOS repair functions—mutations could occur during replication of the plasmid by the 'error-prone' SOS system, whether or not the plasmid has been chemically modified. In this hypothesis, however, the mutation could take place anywhere along the tetracycline gene and not exclusively in the 6S fragment. In fact, we have not found class II mutants in the pool of transformants obtained in the control experiment (that is, ligation of a non-modified 6S fragment).

Nine class II mutant plasmids were isolated from either the wild-type E. coll strain, AB 1157, or the corresponding uvrA mutant strain, AB 1886. The double BamHI-SalI-digested DNA was <sup>32</sup>P-end-labelled at the 5' extremities and sequenced according to the Maxam and Gilbert technique <sup>14</sup>. The sequence of the wild-type 6S fragment of pBR322 was found to be identical to that published by Sutcliffe <sup>15</sup>.

In all of the nine class II mutants we found a mutation located within the 6S fragment. All the mutants showed a deletion of either a single G·C base pair or a doublet of adjacent G·C-C·G base pairs. Two of these mutants (numbers 35 and 36) also had a second mutation (Table 1). Mutants 34 and 36 both exhibit a -2 deletion within the alternating GCGC sequence at positions 435-438 (deletion of a GC sequence at position 435-436 or 437-438 or a CG sequence at position 436-437). (Numbering starts clockwise from the unique EcoRI restriction site.) Moreover, mutant 33 also exhibits a -2 deletion within a GCGC sequence at positions 548-551. Note that the same 6-nucleotide long sequence, GGCGCC, occurs at the site of mutations 34, 36 and 33. The fact that this mutation has been observed three times suggests that the given sequence is a good candidate for a mutational hot spot. Such a GC deletion in an alternating GC

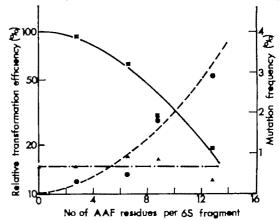


Fig. 2 Relative transformation efficiency and mutation frequencies as a function of the number of AAF residues bound to the 6S fragment. The transformation of E. Coh AB 1157 strain has been performed after UV irradiation of the cells (60 Jm<sup>-2</sup>) as described in Fig. 1 legend. Selection of the transformants was done on LB plates containing ampicillin (50 μg ml<sup>-1</sup>). Mutants were selected by replica plating on LB plates containing tetracycline (20 μg ml<sup>-1</sup>). E. Relative transformation efficiency (log scale), A, class I mutants, Θ, class II mutants

sequence was shown in vivo to be a hot spot for reversion of the mutation, his D3052, in Salmonella by the mutagen 2nitrosofluorene<sup>16</sup>. It is striking that the same type of mutation occurs in both a reversion and forward mutation assay.

As shown in Table 1, four (4, 30, 41 and 45) of the nine mutants show a deletion of a single G residue at position 520 or 521. Note that the four mutants have arisen in different conditions (in two different strains, with or without UV induction of the SOS functions, with very different AAF modification levels). The reason why this particular sequence is a hot spot for mutagenesis is not clear. The possibility that it is a hot spot for the AAF binding reaction itself (the premutational event) is being investigated. Alternatively, it is possible that the processing of the premutational lesions (by the excision repair enzymes for example) is strongly sequence dependent, in which case a hot spot for mutagenesis could arise from the inability of the errorfree repair enzymes to excise the lesion at the given site.

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The mechanism by which single GC base pair deletion mutations occur can be tentatively explained on the basis of the insertion-denaturation model<sup>7,10</sup>, whereby the guanine modified at C-8 with the AAF residue is flipped outside the double helix while the AAF residue is inserted between the two neighbouring base pairs. During replication, the AAF-modified guanine, an essentially non-coding base, blocks the replication machinery17. One can hypothesize that SOS functions then become involved at the replication fork which permit this block to be bypassed by allowing the replication to restart at the next base, thus giving rise to the deletion of the AAF-modified guanine residue.

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## Mechanism of E. coli RecA protein directed strand exchanges in post-replication repair of DNA

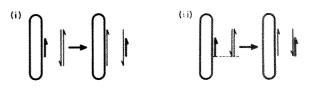
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Escherichia coli mutants carrying recA are both recombination deficient and unable to perform post-replication repair<sup>1,2</sup>. The product of the recA gene regulates the inducible DNA repair functions (the SOS response to DNA damage)<sup>3,4</sup>, and is directly involved in homologous pairing<sup>5-8</sup> and strand exchange<sup>9-12</sup>, two reactions fundamental to recombination and post-replication repair. The filling of post-replication gaps is thought to occur by homologous pairing of the gapped DNA duplex with an intact duplex, followed by cutting of the intact molecule so that sister strand exchanges can take place. Using in vitro systems, we have shown previously that purified RecA protein binds cooperatively to duplex DNA that contains gaps 13, and promotes joint molecule formation (synapsis) between gapped and intact duplexes<sup>7,8</sup>. Moreover, RecA protein promotes a reciprocal exchange of strands between paired DNA molecules 10,12,14. Here, we investigate the mechanism of sister strand exchange thought to occur during post-replication repair. We show that RecA protein initiates strand exchange from a nicked duplex, transferring the 3'-OH terminus at the nick into the single-stranded (ss) region of the gapped molecule. In the presence of ATP, two heteroduplex molecules are formed as RecA protein drives the reciprocal exchanges in one direction starting at the site of the original crossover.

The assay for strand exchange involves the incubation of 32Plabelled duplex restriction fragments with unlabelled circular ssDNA, to which we have annealed a short complementary <sup>3</sup>H-labelled fragment (Fig. 1a(i)). The annealed substrate

#### a DNA substrates that initiate strand exchange



#### b DNA substrates that do not exchange strands

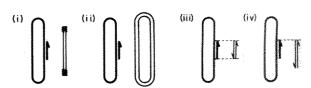


Fig. 1 DNA substrates used in RecA protein-mediated strand exchange tests. The 3' terminus of each strand is indicated with a half arrow. a(i), Annealed substrate (ΦX (+)ssDNA with a 503nucleotide Aval-Avall (-)annealed fragment) and a homologous linear duplex (872-base pair (bp) HaeIII fragment 3) that overhangs both ends of the annealed fragment. These substrates were used in the experiment of Fig. 2. (ii), Homologous duplex that overhangs one end of the annealed fragment (Fig. 2d in ref. 14). Exchange is dependent on polarity and is observed only if the overhanging (-)strand of the duplex has a 3' terminus<sup>14</sup>. b, Strand exchange does not occur when: (i), the duplex has non-homologous ends (wavy lines) (Fig. 2b in ref. 14); (ii), the duplex has no free end (Fig. 1 V in ref. 10); (iii) the duplex is flush-ended with the annealed fragment (Fig. 1 IV in ref. 10); (iv), the (-)strand of the one overhanging end of the duplex has a 5' terminus (Fig. 2c in ref. 14).

sedimented faster through neutral sucrose than the short duplex fragments (Fig. 2a). However, incubation in the presence of RecA protein caused an exchange of labelled strands, heteroduplex DNA being formed as 57% of the <sup>3</sup>H-labelled annealed fragments were transferred from the ssDNA in exchange for  $\sim$ 50% of the (-)strands of the duplex (25% of total  $^{32}$ P label). These results, shown in Fig. 2b, indicate that RecA protein promotes reciprocal strand exchanges to form two heteroduplex molecules and are described in more detail elsewhere 10,14

We wished to test two aspects of RecA protein-mediated strand exchange in relation to the proposed mechanism of post-replication repair<sup>14</sup>. First, does RecA protein initiate strand exchange at a nick in an intact duplex, so transferring a complementary strand into the single-stranded region of a homologous gapped duplex, and second, does RecA protein drive the reciprocal strand exchange in a polar way from the site of the initial crossover. To test these points we constructed the DNA substrates shown in Fig. 3 (i) and (ii).

In a control experiment we reacted the substrates shown in Fig. 3 (i) with RecA protein. The annealed substrate is the same as that used in Fig. 2; it contains both duplex and single-stranded regions and is therefore similar to a gapped duplex. The linear duplex has one end flush with the AvaI end of the annealed fragment and the opposite end is non-homologous with  $\Phi X$  DNA. We have shown previously  $^{10.14}$  that duplexes with ends non-homologous or flush with the annealed fragment do not initiate strand exchanges (Fig. 1b (i) and (iii)). As expected with a duplex of this structure, strand exchanges were not observed (Fig. 3a).

The experiment was then repeated using a similar linear duplex that had been nicked at the BamHI site in one or the other of the two strands (indicated with an arrow in Fig. 3 (i)). Assuming that the site-specific nicking occurred with the same efficiency in both strands, we estimated that  $\sim 20\%$  of the linear fragments used in the reaction mixture contained nicks in the (-)strands. We therefore used an excess of linear duplexes in these experiments. Efficient strand exchanges took place, 41% of the annealed framents being transferred (Fig. 3b).

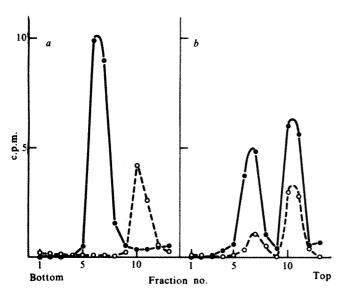


Fig. 2 Neutral sucrose sedimentation profiles showing strand exchanges between the DNA molecules of Fig. 1a(i). Sedimentation is to the left. a, Annealed substrate (unlabelled ΦX ssDNA with <sup>3</sup>H-labelled Aval-AvaII annealed fragment) with <sup>32</sup>Plabelled  $\Phi X$  HaeIII 3 linear duplex; b, same as a but with RecA protein. Annealed substrate, duplex fragments and RecA protein were added where indicated at  $1.75 \,\mu\text{M}$ ,  $0.42 \,\mu\text{M}$  and  $5 \,\mu\text{M}$ , respectively. Duplex fragments and the annealed substrate were prepared as described previously 10 except that AvaI and AvaII were used to digest the <sup>3</sup>H-labelled ΦX RFI DNA for annealed substrate preparation. RecA protein was purified as described elsewhere 13. The standard reaction mixture contained in a total volume of 100  $\mu$ l, 20 mM Tris-HCl pH 7.5, 25 mM MgCl<sub>2</sub>, 2 mM dithiothreitol, 2 mM ATP and 100  $\mu$ g ml<sup>-1</sup> bovine serum albumin. Incubation was for 45 min at 37 °C. The reaction was stopped by the addition of 50 mM EDTA, and proteins were removed by treatment with 200 µg ml<sup>-1</sup> proteinase K and 1% SDS<sup>10</sup>. Centrifugations were through 5 ml of 5-20% neutral sucrose in a Beckman SW 50.1 rotor at 45,000 r.p.m. for 180 min at 4 °C. Fractions collected from the gradients were counted for radioactivity in Formula 963 scintillation fluid (NEN). lacktriangle,  $^3H$ ,  $\times 10^{-2}$ ;  $\bigcirc$ ,  $^{32}P$ ,

Table 1 Tests for strand exchange between the annealed substrate and a nicked duplex: the effect of nick closing by T4 DNA ligase

Linear duplex a, Nicked chimaeric duplex (Fig. 3 (i))	Ligase	RecA +	% Strand exchange 33
b, Same as a	+	+	8
c, Homologous duplex (ΦΧ HaeIII-AvaI)	-	+	40
d, Same as c	+	+	40

The reactions described here were carried out in two stages, ligation and then strand exchange. In the first, the duplex fragments, either 0.42 μM ΦX linear HaeIII-AvaI or 3.15 μM nicked pSW1 HaeII-Aval, were incubated for 20 min at 25 °C with or without 8×10 of T4 DNA ligase (BRL) in 100 µl of 50 mM Tris-HCl pH 7.8, 10 mM MgCl<sub>2</sub>, 2 mM dithiothreitol, 1 mM ATP and 50 µg ml 1 bovine serum albumin. The reaction mixtures were heated for 5 min at 60 °C to inactivate the ligase, and cooled to ice temperature. In the second stage, we added 100  $\mu$ l of buffer containing 20 mM Tris-HCl pH 7.5, 25 mM MgCl<sub>2</sub>, 2 mM dithiothreitol, 2 mM ATP, 100  $\mu$ g ml<sup>-1</sup> bovine serum albumin, 1.75 µM of the annealed substrate (Fig. 1a (i)) and 5 µM RecA protein, and incubated the mixtures for 45 min at 37 °C. Proteins were removed from the DNA and the reaction products sedimented through neutral sucrose as described in Fig. 2 legend. The linear duplex  $\Phi X$  HaeIII-AvaI was prepared as described previously 14 and the nicked duplex was prepared as in Fig. 3 legend. The per cent strand exchange was calculated from the amount of total <sup>3</sup>H-labelled annealed fragments that were displaced from the annealed substrate. The concentration of DNA ligase used here was predetermined as an amount sufficient to ligate 80% (as determined by alkaline sucrose sedimentation) of the nicks in 100 µl of 3.5 µM pBR322 DNA. This DNA had been nicked by pancreatic DNase I in the presence of ethidium bromide. The conditions of the experiment did not result in any dimerization of the linear duplex fragments (detected by polyacrylamide gel electrophoresis).

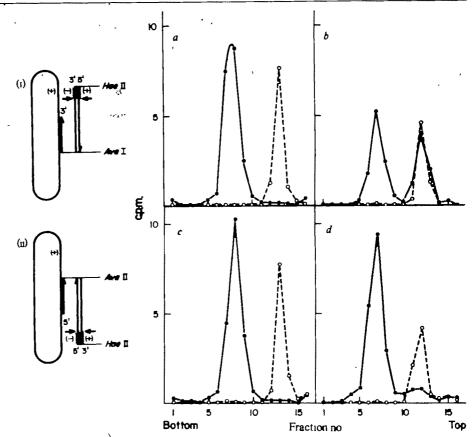
To confirm that strand exchanges were indeed initiated from the nick in the duplex molecule, we incubated the nicked DNA with T4 DNA ligase just before the addition of RecA protein and the annealed substrate to the reaction mixture. RecA protein did not promote strand exchanges when the nicks were preligated (Table 1b), whereas incubation of an identical reaction mixture without DNA ligase resulted in efficient strand exchanges (Table 1a). In a control to test whether RecAmediated strand exchanges might be inhibited by the presence of DNA ligase, we examined the effect of ligase on a similar reaction between the annealed substrate and a linear duplex. The results in Table 1c and d show that strand exchanges occurred with the same efficiency in the absence or presence of DNA ligase, indicating that no inhibitory factors were present in the reaction mixture.

These results show that strand exchanges take place efficiently between an annealed substrate and a duplex nicked at the BamHI site (Fig. 3 (i)). The nick was essential for the initiation of strand exchanges.

It is interesting that the 3'-terminal base of the strand transferred from the nicked duplex is derived from a molecular linker inserted into the plasmid pSW1 during construction of the chimaeric plasmid  $^{14}$ . The terminus ending CCG-3'OH is transferred to the complementary  $\Phi X$  sequence... GGT....5' to form a G/T single nucleotide mismatch (sequence from Sanger et al. 15). Thus, although this single nucleotide mismatch at the 3' terminus does not block strand exchange, we have shown previously that a non-homologous terminal region of 150 bases is sufficient to block the reaction  $^{14}$ .

To test whether strand exchanges from a nick are driven in a polar way, we incubated the DNA substrates shown in Fig. 3 (ii) with RecA protein. These substrates are similar to those described above (Fig. 3 (i)) except that the BamHI nick of the duplex is in a different position relative to the annealed fragment. The results shown in Fig. 3d indicate that this nicked duplex is inefficient at strand exchange,  $\sim$ 9% of the <sup>3</sup>H-labelled annealed fragments being transferred. In the absence of a nick,

Fig. 3 Neutral sucrose sedimentation profiles to show initiation of strand exchange by the nicked duplexes shown in (i) and (ii). a, Annealed substrate with intact Haell-Apal duplex DNA and RecA protein. b, Same as a but the duplex was nicked at the BamHI site. c, Annealed substrate with intact AvaII-HaeII duplex DNA and RecA protein. d, Same as c but the duplex was nicked at the BamHI site. In substrates (i) and (ii), nonhomologous DNA is indicated by a wavy line, and the site of nicking by BamHI/EtBr is arrowed. In all reactions, the annealed substrate, duplex fragments and RecA protein were present at 1.75 µM, 3.15 µM and 5 µM, respectively. Incubation and sedimentation were as described in Fig. 2 legend. Duplex fragments were prepared by digesting pSW1 form I <sup>32</sup>P-labelled DNA with *Hae*II and fragment 3 was purified <sup>14</sup>. The fragment was extracted with isobutanol to remove ethidium bromide and digested with either AvaI or AvaII. The larger fragments in each case were purified and extracted with phenol and isobutanol. Nicked duplex fragments were prepared in the same way from pSW1 DNA that



was nicked by Bami in the presence of ethicium bromide<sup>21</sup>. As 80% of the closed circles were nicked in the BamHI/EtBr reaction (determined by agarose gel electrophoresis and alkaline sucrose sedimentation), and as the single nick could be in either strand at one of the two BamHI sites, we estimate that 20% of the duplexes contained nicks in the (-)strand at the BamHI site.  $\bullet$ ,  ${}^{3}$ H,  $\times 10^{-2}$ ;  $\circ$ ,  ${}^{32}$ P,  $\times 10^{-2}$ .

we found the normal background (<5%), indicating that no strand exchange had taken place (Fig. 3c). Comparison of the data presented in Fig. 3b and d indicates that RecA protein shows a strong directional bias for the extension of the heteroduplex region from the nick. As the polarity of each DNA strand is known from the DNA sequence 15, the directionality of

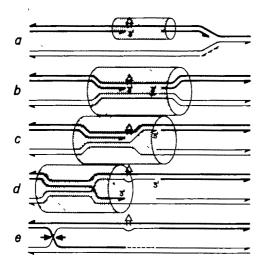


Fig. 4 Model for the repair of a post-replication gap by RecA protein-mediated sister strand exchanges. RecA protein molecules (shaded area) bind at the post-replication gap (a) promoting homologous pairing (b). The intact molecule is nicked by the cutting in trans activity (b) and the 3' terminus of the nicked strand is transferred by RecA protein into the post-replication gap (c). Directional strand transfer driven by RecA protein results in the reciprocal transfer of the 3' terminus of the gapped strand to produce a crossed strand exchange or Holliday structure (d). The crossed strand exchange is cut leading to completion of the repair (e).

RecA-driven exchanges can be determined. The efficient strand exchange initiated by the nicked duplex (Fig. 3 (i)) occurs by transfer of the free 3'-OH terminus at the nick of the complementary strand into the single-stranded region of the annealed substrate. The heteroduplex region is then extended by RecA protein transferring strands in a 3' to 5' direction (relative to the transferred strand). The polarity of strand exchange shown here is therefore similar to those involving exchanges initiated by a linear duplex14,16,17

The experiments described here suggest a way in which RecA protein may exchange DNA strands in post-replication repair. As shown in Fig. 4, DNA polymerase moving in a 3' to 5' direction along the template strand is blocked at a pyrimidine dimer and reinitiates synthesis further along the chain. The daughter strand is therefore terminated with a 3'-OH at the position of the dimer (Fig. 4a). The resulting ssDNA activates the RecA protease 18, triggering the SOS response and increasing the synthesis of RecA protein in readiness for pairing with the intact sister duplex (Fig. 4b). Pairing may occur at the site of the gap with the single-stranded region of the gapped molecule wound in the major groove of the duplex. Once homologous contacts are established, a cutting in trans enzyme may nick the intact sister duplex at a site opposite the gap 19 (Fig. 4b). The nick enables RecA protein to transfer the 3'-OH terminus of the nicked strand into the gap, thus providing an intact strand complementary to the one containing the dimer (Fig. 4c). The strand exchange reaction is therefore initiated in a threestranded way as RecA protein drives branch migration and heteroduplex formation beyond the dimer. The 3'-OH terminus of the gapped strand is also transferred, producing a crossed strand exchange (Fig. 4d). From that point on, the four strands are paired and exchanged as described previously10 to produce duplex molecules. Both helices can now be repaired by DNA polymerase I and ligase (Fig. 4e) and completion of repair permits the release of RecA protein and terminates the SOS response. The resulting crossed strand exchange is presumably

resolved by a further recombination enzyme, perhaps similar to the phage T4 gene 49 nuclease<sup>20</sup>. The final structure retains the pyrimidine dimer, but now has an intact complementary strand and can be repaired by excision enzymes

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#### Effect of base-pair stability on the melting of superhelical DNA

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It is well established that the structure 1,2 as well as the stability of the DNA duplex is affected by supercoiling. Supercoiled DNA has been shown to possess increased stability and is thus characterized by an elevated melting temperature as well as a broader melting transition. Thermal melting analysis has been the most widely used method for studying the structural and dynamic properties of complex DNA species. Here we report a remarkable attribute of supercoiled DNA, that the width of the melting transition of superhelical DNA does not depend appreciably on

the relative stabilities of its A·T and G·C base pairs.

It has been shown earlier<sup>3.4</sup> that superhelical DNA (DNA I) melts at higher temperatures and over a broader temperature range than do open circular molecules (DNA II). These experiments were carried out in conditions such that the stabilities of the A·T and G·C base pairs were substantially different. To eliminate the possible influence of this factor on the melting process, we performed the thermal denaturation of DNA in experimental conditions in which the A·T and G·C base pairs were of equal stability by dissolving the DNA in a concentrated solution of tetraethylammonium bromide (TEA)5

Figure 1a shows the integral and differential melting curves of the replicative form of phage  $\Phi X174$  DNA in a solution containing 3 M TEA (the sample was a 10:1 mixture of DNA I and DNA II). The step at 58 °C in the melting curve (the sharp peak in the differential curve) corresponds to the melting of DNA II. Its melting range is less than 1 °C wide, thus indicating that the thermal stabilities of A T and G C base pairs are approximately equal5,

The melting of DNA I seems to be quite different. Its first intriguing feature is the width of the denaturation range. Melting is appreciable at 60 °C but even at 95 °C ~10% of the base pairs are still in the native state. Over the temperature range 60-90 °C the derivative  $d\theta/dT$  increases and reaches  $1/25\,^{\circ}\mathrm{C}$  at the transition midpoint ( $\theta = 1/2$ ).

Figure 1b shows the melting curves of the same sample as in Fig. 1a, but in a solution containing 7.3 M NaClO<sub>4</sub> (conditions in which the difference in stability between A·T and G·C base pairs,  $T_{GC} - T_{AT}$  is ~56 °C)<sup>7</sup>. Here DNA II melting occurs over a broad (8-10 °C) range, and the differential curve shows a resolved fine structure.

In contrast, the melting profile of DNA I remains practically unchanged, and the derivative  $d\theta/dT$  at the transition midpoint has the same value of 1/25 °C. Moreover, the temperature difference between the melting midpoints of DNA I and DNA II is the same (25 °C) in both experimental situations (Fig. 1a, b). These findings elucidate the fundamental property of superhelical DNA-that, in contrast to linear DNA or DNA II, its melting is insensitive to the value of  $T_{\rm GC}-T_{\rm AT}$  and, therefore, to the nucleotide composition heterogeneity.

A slight increase of absorbance before the melting of DNA II can be seen in both Fig. 1a and b. In Fig. 1a this effect is not superimposed on the melting of DNA II and its magnitude can be evaluated ( $\theta \approx 0.05$ ); it corresponds roughly to the degree of denaturation necessary to remove all superhelical turns (as reported elsewhere<sup>8</sup> the superhelical density of ΦX174 DNA is 0.063). Thus, the increase in absorbance could be attributed to early melting of DNA I3. However, this effect is of the same order of magnitude as the baseline instability of the instrument, further investigation is necessary.

The theory of the 'helix-coil' transition in polynucleotides without topological constraints (linear or open circular DNAs) is

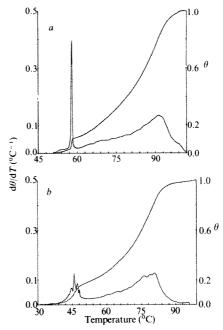


Fig. 1 The integral and differential melting curves of the replicative form of phage  $\Phi$ X174 DNA; a, in the presence of 3 M TEA  $(T_{GC} - T_{AT} \approx 0 \, ^{\circ}\text{C})^{5.6}$ , b, in the presence of 7.3 M NaClO<sub>4</sub>  $(T_{GC} - T_{AT})$  $(T_{\rm GC}-T_{\rm AT}\approx0~{\rm ^{\circ}C})^{5.6}$ , b, in the presence of 7.3 M NaClO<sub>4</sub>  $(T_{\rm GC}-T_{\rm AT}\approx56~{\rm ^{\circ}C})^{7}$ . A 120-µl aliquot of solution contained 10-12 µg ml<sup>-1</sup> DNA (10:1 mixture of DNA I and DNA II), 10 mM phosphate buffer pH 8.0 and the concentration of TEA or NaClO<sub>4</sub> indicated above. The melting curves were registered on a Carry 218 spectrophotometer, equipped with a specially constructed sample holder with a facility for temperature control. Temperature scanning rate was  $0.2\,^{\circ}\text{C}$  min $^{-1}$ . The melting curves were differentiated using a Hewlett-Packard 9830A computer.

well developed, and excellent agreement with experimental data has been achieved for such molecules7. However, the abovementioned features of the melting of covalently closed DNA cannot be explained within the framework of this theory. An adequate explanation should be based on a correct account of the effect of topological constraints on the thermodynamical properties of DNA.

Despite the disruption of the double helix, the topological state of DNA I (linking number of the strands, Lk) remains invariable. That leads to an excess of Lk and therefore to extra free energy in DNA I compared with DNA II at the same degree of denaturation  $(\Delta Lk \sim \theta)$ .

When DNA is treated as a thin ribbon (an approximation which seems to be reasonable at small degrees of denaturation), a part of Lk can be isolated (writhing, Wr) which is determined by the spatial configuration of DNA duplex axis only. Then the difference (Lk - Wr) will account for the twist (Tw) of the ribbon9. The extra free energy of DNA I within the melting interval is a function of both Wr and Tw. These values cannot be precisely estimated at present. In this situation a heuristic approach seems to be reasonable. In the most advanced theoretical work on the problem<sup>10</sup> it was assumed that the strands in the melted region remain twisted to the same extent as before denaturation. Thus, the possible contribution of Wr to Lk was ignored. According to this model, the melting range for DNA I should be 2-3 times larger than that for DNA II<sup>10</sup>. The latter factor fits the experimental data when the value of  $T_{\rm GC} - T_{\rm AT}$  is large. However, for  $T_{\rm GC} \simeq T_{\rm AT}$  there is marked disagreement between calculated and experimentally obtained values for the width of melting range

We consider the failure of the model to be due to the neglect of the possible contribution of Wr to Lk. Because of the enhanced flexural 'softness' of partially melted DNA the latter can be significant. The theoretical treatment of the model, allowing for such a possibility, will be reported elsewhere.

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## Polycythaemia- and anaemia-inducing strains of spleen focus-forming virus differ in post-translational processing of envelope-related glycoproteins

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A virus preparation was isolated by Friend which produced a rapid erythroblastosis in certain strains of mice1. Although the original virus preparation induced a slight anaemia in the terminal stages of the disease, other variants, derived from various in vivo and in vitro passages of the original virus, were associated with polycythaemia<sup>2,3</sup>, and the different viruses were accordingly classified as FVA (Friend virus anaemia-inducing) or FVP (Friend virus polycythaemia-inducing). Another important difference between the two strains is that whereas FVP induces in infected mice cells that are capable of forming

colonies in vitro in the absence of erythropoietin4.5, colonies formed by cells derived from FVA-infected mice are erythropoietin dependent<sup>6-9</sup>. Differences were also noted in the amount of haemoglobin present in erythroid bursts produced after in vitro infection of haematopoietic cells with FVP or FVA<sup>22</sup>. Both FVA and FVP virus stocks have been shown to consist of at least two different viruses: the spleen focus-forming virus (SFFV<sub>P</sub> or SFFV<sub>A</sub>), a replication defective virus; and the Friend murine leukaemia virus (F-MuLV), a replication competent virus which acts as a helper virus for replication of the defective SFFV<sup>6,23</sup>. Here, in an attempt to correlate differences in the biological effects of the SFFVs derived from FVP and FVA stocks with biochemical properties of proteins encoded by them, we have compared the post-translational processing of the envelope glycoproteins encoded by these viruses, and have found both qualitative and quantitative differences in the processing of the envelope glycoproteins of the two virus stocks.

Although the genomes of the SFFVs derived from FVP and FVA stocks differ slightly<sup>10</sup>, both encode similar primary translational products—a 45,000 molecular weight (M<sub>r</sub>) gag-related protein (p45gag) and a 52,000-Mr envelope-related glycoprotein (gp52<sup>env</sup>)<sup>6,11,12</sup>. Studies with subgenomic fragments of molecularly cloned SFFV<sub>P</sub> have shown that the biological activity of SFFV requires<sup>13</sup> the gene encoding gp52<sup>env</sup>, but not that encoding p45gag. Differences in the tryptic peptides of gp52env exist among different strains of SFFV<sub>P</sub> (S.K.R., unpublished data) as well as between the SFFVs derived from FVP and FVA stocks6. However, these differences have not been correlated with the different biological effects of SFFV<sub>P</sub> and SFFV<sub>A</sub>.

When NRK fibroblasts were nonproductively infected with the Lilly-Steeves strain of SFFV<sub>P</sub> (ref. 14) and the major product of the *env* gene detected by pulse-labelling the cells with <sup>35</sup>S-methionine and immune precipitating with specific anti

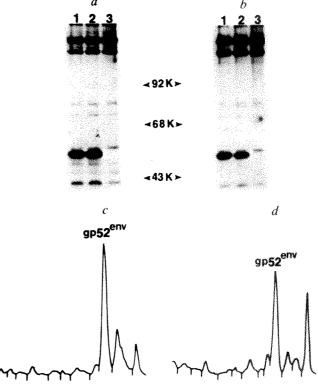


Fig. 1 Immune precipitation of <sup>35</sup>S-methionine-labelled SFFV<sub>P</sub> and SFFV<sub>A</sub> cytosol proteins. Fibroblasts nonproductively infected with either SFFV<sub>P</sub> (a) or SFFV<sub>A</sub> (b) were labelled for 30 min with  $^{35}$ C and  $^{35}$ C. S-methionine and then immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes 1), a goat antiserum specific for the gp70 of MCF murine leukaemia viruses (lanes 2), or normal goat serum (lanes 3). Precipitates were then electrophoresed on 7% SDS-polyagralamida and % SDS-polyacrylamide gels and exposed to X-ray film as previously described 12. c, d Represent densitometer scans of lanes 1 in a and b, respectively.

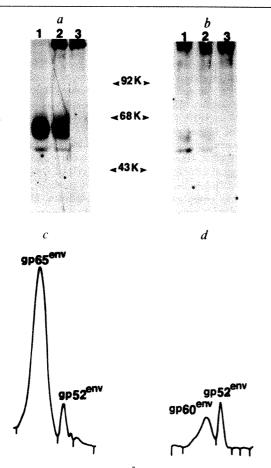


Fig. 2 Immune precipitation of  ${}^{3}$ H-galactose-labelled SFFV<sub>p</sub> and SFFV<sub>A</sub> cytosol proteins. Fibroblasts nonproductively infected with either SFFV<sub>p</sub> (a) or SFFV<sub>A</sub> (b) were labelled with  ${}^{3}$ H-galactose and then immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes 1), a goat antiserum specific for the gp70 of MCF murine leukaemia viruses (lanes 2), or normal goat serum (lanes 3). The precipitates were processed as described in Fig. 1 legend. c, d Represent densitometer scans of lanes 1 in a and b, respectively.

gp70 sera as previously described<sup>12</sup>, a  $52,000-M_r$  protein (gp52<sup>env</sup>) was specifically precipitated (Fig. 1a, lanes 1, 2). Post-translational processing of the SFFV envelope proteins and expression of these proteins at the cell surface were then studied by pulsing the cells with <sup>3</sup>H-galactose<sup>15</sup> or labelling the cells with <sup>125</sup>I in the presence of lactoperoxidase<sup>12</sup>, respectively, before immune precipitation with specific antisera. Proteins precipitated in each case were visualized by autoradiography after electrophoresis on SDS-polyacrylamide gels. When SFFV<sub>P</sub>/NRK cells were labelled with <sup>3</sup>H-galactose, gp52<sup>env</sup> and 65,000-M<sub>r</sub> glycoprotein (gp65<sup>env</sup>) were detected, indicating that the gp52<sup>env</sup> is processed by the addition of complex carbois processed by the addition of complex carbohydrates to a higher molecular weight form (Fig. 2a, lanes 1, 2). gp65<sup>env</sup> was detected on the cell surface when cells were labelled with <sup>125</sup>I in the presence of lactoperoxidase (Fig. 3a, lanes 1, 2). This seems to be a common pathway for SFFV<sub>P</sub> envelope proteins, as two other polycythaemia-inducing strains of SFFV, the Mirand and Axelrad strains, synthesize 62-68,000-M<sub>r</sub> envelope glycoproteins which can be detected after metabolic labelling of nonproducer cells with <sup>3</sup>H-galactose<sup>15</sup> face labelling with 125 I (S.K.R., unpublished data).

Cells nonproductively infected with SFFV derived from an anaemia-inducing stock of Friend virus<sup>11</sup> also synthesized a 52,000-M<sub>r</sub>, glycoprotein that could be labelled with <sup>35</sup>S-methionine and immune precipitated with anti-gp70 sera (Fig. 1b, lanes 1, 2). When SFFV<sub>A</sub> nonproducer cells were labelled with <sup>3</sup>H-galactose, gp52<sup>env</sup> but no gp65<sup>env</sup> was detected (Fig. 2b, lanes 1, 2). However, the cells contained a low amount of a 60,000-M<sub>r</sub>, glycoprotein that was precipitable with anti-gp70 sera. When we attempted to label surface proteins

with <sup>125</sup>I and lactoperoxidase, no envelope-related proteins were detected on the surface of SFFV<sub>A</sub> nonproducer cells (Fig. 3b, lanes 1, 2).

The differences in the levels of processed envelope glycoproteins in SFFV<sub>P</sub> and SFFV<sub>A</sub> nonproducer cells were quantified by scanning films of the gels with a densitometer. Cells nonproductively infected with either expressed almost equivalent levels of gp52<sup>env</sup> (Fig. 1, 2, panels c, d) but SFFV<sub>P</sub> nonproducer cells expressed eightfold higher levels of any high molecular weight, galactose-containing envelope glycoprotein than did SFFV<sub>A</sub> nonproducer sells (Fig. 2c, d).

Differences in processing of SSFV<sub>P</sub> and SFFV<sub>A</sub> gp52<sup>env</sup> were also detected *in vivo*. Spleens of mice infected with either the FVP complex (SFFV<sub>P</sub> pseudotyped with F-MuLV) or the FVA complex (SFFV<sub>A</sub> pseudotyped with F-MuLV) were labelled with either <sup>35</sup>S-methionine or <sup>3</sup>H-galactose and immune precipitated with anti-gp70 sera. F-MuLV and SFFV envelope glycoproteins could be distinguished from each other using a MCF-specific antiserum that will precipitate SFFV, but not F-MuLV, envelope glycoproteins<sup>12</sup>. As shown in Fig. 4, spleens from mice infected with either virus expressed equivalent amounts of SFFV gp52<sup>env</sup> (Fig. 4a, lanes 1, 2; b, lanes 1, 2) but differed in the levels of the processed form of gp52<sup>env</sup> detected by labelling with <sup>3</sup>H-galactose (Fig. 4c, lanes 1, 2; d, lanes 1, 2). Equivalent levels of <sup>35</sup>S-methionine- and <sup>3</sup>H-galactose-labelled F-MuLV-encoded envelope proteins were detected in spleens from animals infected with either virus.

Our results indicate that the processing of the envelope glycoproteins encoded by the SFFVs derived from FVP and FVA stocks differs both quantitatively and qualitatively. The gp52<sup>env</sup> encoded by three different strains of SFFV<sub>P</sub> is processed to a galactose-containing 65,000- $M_r$  glycoprotein which eventually appears on the cell surface, whereas most of the gp52<sup>env</sup> encoded by SFFV<sub>A</sub> is not further processed to a higher molecular weight, galactose-containing form and cannot be detected on the cell surface. Cells nonproductively infected with the Rauscher strain of SFFV, another anaemia-inducing erythroblastosis virus<sup>16</sup>, show similar differences in envelope glycoprotein processing and transport.

A variety of membrane glycoproteins have been shown to contain a signal peptide at their amino terminus that is believed to be necessary for proper processing of the glycoproteins and transport to the cell surface<sup>17-19</sup>. Although such a signal peptide has not been identified for any of the envelope proteins of RNA tumour viruses, one could postulate that the difference in the processing of SFFV<sub>P</sub> and SFFV<sub>A</sub> envelope proteins is due to a

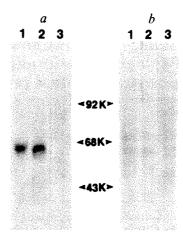


Fig. 3 Immune precipitation of  $^{125}$ I-labelled SFFV<sub>P</sub> and SFFV<sub>A</sub> cell-surface proteins. The surface proteins of cells nonproductively infected with either SFFV<sub>P</sub> (a) or SFFV<sub>A</sub> (b) were labelled with  $^{125}$ I in the presence of lactoperoxidase and then immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes 1), goat antiserum specific for the gp70 of MCF murine leukaemia viruses (lanes 2), or normal goat serum (lanes 3). The precipitates were processed as described in Fig. 1 legend.

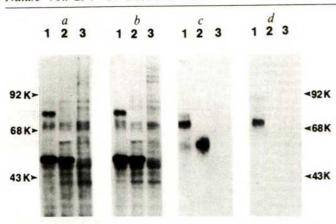


Fig. 4 Immune precipitation of spleen cell extracts from mice infected with either the FVP or FVA complex. Spleen cells from BALB/c mice infected 21 days previously with either 4×10 focus-forming units of F-MuLV/SFFV<sub>P</sub> (a, c) or  $1-2 \times 10^4$  focusforming units of F-MuLV/SFFV<sub>A</sub> (b,d) were labelled with either <sup>35</sup>S-methionine (a,b) or <sup>3</sup>H-galactose (c,d) and then immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes 1), a goat antiserum specific for the gp70 of MCF murine leukaemia viruses (lanes 2), or normal goat serum (lanes 3). Precipitates were processed as described in Fig. 1 legend.

mutation in the gene encoding the signal peptide of the SFFVA gp52env, resulting in defective processing and failure to transport the protein to the cell surface. Consistent with such a hypothesis, oligonucleotide fingerprinting data show that the only detectable differences in the RNAs of FVP and FVA strains of SFFV are six oligonucleotides at the extreme 5' end of the RNA and two oligonucleotides at the 5' end of the envelope gene that are missing in SFFV<sub>P</sub> RNA as well as the RNAs of F-MuLV and Friend MCF virus 10. As the envelope message of RNA tumour viruses has been shown to contain sequences spliced from the 5' end of the genome<sup>20,21</sup>, a signal sequence for the envelope gene of SFFV could be present in either of the areas of the SFFV genome which are different in SFFV<sub>P</sub> and SFFV<sub>A</sub>.

The differences in the processing of envelope glycoproteins encoded by SFFV<sub>P</sub> and SFFV<sub>A</sub> may be responsible for the different biological activities of these viruses. For example, the concentration of gp65<sup>env</sup> on the cell surface could be important in determining the ability of proliferating cells to differentiate in the presence of various concentrations of erythropoietin. Alternatively, differences in biological activity and glycosylation could be independent consequences of a difference in the primary amino acid sequence of the envelope glycoproteins. Analysis of the biological effects of SFFV<sub>P</sub> in conditions where glycoprotein processing is inhibited, sequencing of the envelope genes of molecularly cloned DNAs from SFFV<sub>P</sub> and SFFV<sub>A</sub>, and generation of mutants of molecularly cloned SFFV<sub>P</sub> that biologically resemble SFFVA should help to establish a relationship between the observed differences in glycoprotein processing and the different biological effects of these SFFV variants.

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### Haemolysin contributes to virulence of extra-intestinal E. coli infections

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Escherichia coli is the predominant facultative microorganism isolated from the gastrointestinal tract of man and is the most common enteric organism causing extra-intestinal infections in man, particularly of the urinary tract, peritoneum and blood 1,2. It is likely that a consortium of virulence factors is responsible for the initiation and severity of extra-intestinal E. coli infections. Properties reported to be associated with the virulence of such infections include haemolysin production3, K1 antigen production<sup>4,5</sup>, various O antigens<sup>5</sup> and Fe sequestration6. For example, it has long been recognized that the ability to lyse erythrocytes is a phenotype more common to E. coli strains isolated from infections than those found in normal faeces 3,7-9. It is not clear whether the haemolysin per se is a virulence determinant. However, here we report that an isolated DNA sequence encoding haemolysin, added by recombinant DNA technology to avirulent non-haemolytic faecal isolates of E. coli, results in strains having enhanced virulence as measured in an experimental rat peritonitis model.

Several laboratories have demonstrated that the haemolysins commonly seen among animal faecal isolates of E. coli are encoded by transferable plasmids 10-12. There is evidence that these large plasmids confer virulence when transferred to avirulent strains of E. coli12. However, it is not known whether the haemolysin itself or another unrecognized plasmid-mediated gene is responsible for this virulence phenomenon. Goebel and co-workers have demonstrated using DNA · RNA hybridization that plasmid-mediated haemolysin gene sequences are generally homologous with one another<sup>13</sup>. However, we have found that the haemolysin gene(s) of human E. coli clinical isolates most often resides on the bacterial chromosome14 Recently, chromosomal and plasmid-encoding haemolysin genes have been shown to share regions of DNA sequence homology, suggesting a common origin15

We have previously shown 14 by bacterial mating experiments that the haemolysin locus of the E. coli J96 isolate that causes human urinary tract infection resides on the bacterial chromosome. By cosmid cloning16, we have constructed a library of gene sequences of this organism in E. coli K-12. From a haemolytic E. coli K-12 clone we have identified and subcloned an 11.7-kilobase (kb) SalI restriction endonuclease DNA fragment encoding haemolysin and introduced this DNA fragment into the recombinant DNA vector pACYC184, resulting in a hybrid plasmid, pSF400017. Using the ampicillinresistance transposon Tn1 as a site-specific mutagen<sup>18</sup>, together with an in vitro-derived deletion, we have localized the haemolysin gene sequence to a 7.5-kb portion of pSF400019.

Table 1 Effect of cloned haemolysin gene sequences on rat mortality in experimental intra-abdominal infections

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Strain	Source	Haemolytic	Plasmid content	Rat deaths*/rats inoculated	
J198	Human faeces	Name.	Name .	3/32	
WAF 107	Transformation of J198	+	pSF4000	26/28	
WAF 108	Transformation of J198	anaga.	pSF4000::Tn1	0/20	
WAF 111	Transformation of J198	+	pAN202-312	1/19	

A single cryptic plasmid species is present in J198, which is colicin V-negative (ColV<sup>-</sup>).

\* Number of deaths occurring within 7 days after receiving intraperitoneal injection of  $10^7$  colony-forming units of each strain. Inocula were suspended in 10% barium sulphate (w/v)/50% sterile human faeces in brain heart infusion broth. Volume of inoculum in each case was 0.25 ml.

Figure 1 shows a restriction endonuclease fragment map of the haemolysin-encoding region of pSF4000.

The common laboratory strain of E. coli K-12 is considered to be an attenuated organism which cannot be converted to a virulent state by the acquisition of single virulence determinants<sup>4,5</sup>. Therefore, appropriate testing of putative virulence properties should be done in the genetic background of 'wild' strains of E. coli represented by faecal isolates from healthy individuals. From our strain collection, a non-haemolytic 'wild' strain (J198) has proved useful because it can be transformed with plasmid DNA by the CaCl/cold shock technique of Lederberg and Cohen<sup>20</sup>. This particular strain was also attractive for pathogenesis studies because we had found it to be avirulent for rats in an experimental intra-abdominal sepsis

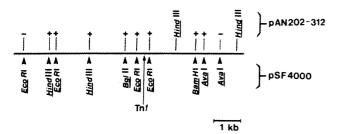


Fig. 1 Physical map of the haemolysin-encoding region of pSF4000. Restriction endonuclease cleavage sites for pSF4000 are shown below the bar. The pSF4000 plasmid was compared with pAN202-312 by restriction endonuclease fragment analysis. pAN202-312 is a recombinant plasmid (vector pACYC184) containing the haemolysin sequence derived from pHly152 (W. Goebel, personal communication); pHly152 was isolated from an E. coli strain found in mouse faeces 13. The restriction sites common to both pSF4000 and pAN202-312 are designated by +. Restriction sites present in pSF4000 but absent from pAN202-312 are indicated by -. Two HindIII sites present in pAN202-312 but absent from pSF4000 are shown above the bar.

model<sup>21</sup>. This model mimics in two stages the disease process of peritonitis and multiple abscess formation seen in humans suffering intra-abdominal sepsis<sup>21</sup>. During the initial acute peritonitis phase in the rat model there is a high mortality rate associated with bacteraemic infections by E. coli or enterococci; here we have focused our attention on the role of putative E. coli virulence factors during this initial phase. The data presented in Table 1 illustrate a significant enhancement in rat mortality when pSF4000 is introduced into J198, which suggests that the haemolysin encoded by pSF4000 is responsible for the lethal behaviour of these strains in this animal model. In support of this, a Tn1 insertional mutant of pSF4000 which no longer confers the haemolysin phenotype does not confer virulence to J198 when present in the same genetic background. The particular Tn 1 insertion used in the virulence studies (see Fig. 1) is in the region which encodes the structural gene for the haemolysin of pHly152 (ref. 22; R.A.W. and S.F., unpublished observations). The observation that the cloned haemolysin derivative pAN202-312 does not confer the same degree of virulence for the rat on J198 as pSF4000 is an interesting finding in view of their apparent sequence homology (Fig. 1). These data illustrate that similarity in phenotype and even close genetic homology may not be sufficient to engender virulence in a particular microorganism. Whether this reflects the structural differences between the 'human' and 'animal' haemolysins or the route by which the infecting microorganism is introduced into a susceptible host awaits further study. Recently, we have observed that another cloned haemolysin of animal origin confers slightly greater virulence than that of pAN202-312 in the rat model (R.A.W., E.P.D. and S.F., unpublished observations). These differences may lead to better understanding of the molecular basis of haemolysin action.

It was of interest to measure the level of virulence of our in vitro-derived pathogen (WAF 107) compared with the original clinical isolate J96 from which the haemolysin gene sequence was taken. It is apparent that the virulence of these two strains are similar quantitatively (Table 2). However, we are hesitant to conclude that acquisition of the haemolysin converts avirulent faecal strains into virulent organisms typical of those found in extra-intestinal infections for two reasons. First, we have noted that the course of infection differs for the two strains. Rats infected with J96 become symptomatic sooner and die earlier than those infected with WAF 107. In most cases, J96-infected rats died 12-24 h post-injection whereas most WAF 107infected rats died between 24 and 36 h after infection. Second, there may be a significant difference in haemolysin gene dosage between clinical isolates and WAF 107 as our haemolysin gene sequence was cloned into a multi-copy plasmid vector. The significance of the dosage effect awaits further experimentation. Table 2 also shows representative LD<sub>50</sub> data for an E. coli K-12 prototroph harbouring the pSF4000 plasmid, which support the observations of other laboratories<sup>4.5</sup> that the virulence of *E. coli* K-12 can be enhanced only slightly by the acquisition of any particular virulence property.

As E. coli infections of the urinary tract and other extraintestinal sites are among the most common of human bacterial diseases, there is wide interest in understanding the precise mechanisms which contribute to the pathogenesis of these infections. Recombinant DNA technology represents a means of measuring the contribution of individual determinants of pathogenicity which are part of a polygenic virulence phenomenon. Although the precise mode of action of E. coli haemolysin in the pathogenesis of infection is still unclear, our data show that it has a significant role.

**Table 2** LD<sub>50</sub> of selected strains in experimental rat intra-abdominal sepsis

Strain	Characteristics	LD <sub>50</sub>
J96	O4, ColV <sup>+</sup> , hly <sup>+</sup> , Hah <sup>+</sup>	$3.5 \times 10^{5}$
J198	O22, ColV, hly, Hah	$>10^{7}$
WAF 107	J198 transformed with pSF4000 DNA*	$1.6 \times 10^{5}$
W1485	K-12 prototroph, ColV, hly, Hah	$>10^{7}$
WAF 109	W1485 transformed with pSF4000 DNA,	$1\times10^7$
	ColV, hly, Hah	

The method of Reed and Muench<sup>23</sup> was used to determine the lethal dose for 50% of the population,  $LD_{50}$ .  $ColV^+$ , colicin V production;  $hly^+$ , haemolytic;  $Hah^+$ , haemagglutination of human erythrocytes.

\* pSF4000 encodes a haemolysin cloned from the J96 background.

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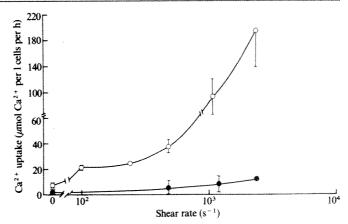
## Physiological shear stresses enhance the Ca2+ permeability of human erythrocytes

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A membrane-bound  $Ca^{2+}$  pump, capable of extruding  $Ca^{2+}$  at a rate of  $4-30\times 10^{-3}$  mol  $Ca^{2+}$  per l of cells per h in optimal conditions<sup>1</sup>, enables mammalian erythrocytes to maintain a large transmembrane Ca2+ concentration gradient (10-3M outside compared with 10<sup>-6</sup>M inside)<sup>2</sup>. This rate is much greater than the passive  $Ca^{2+}$  influx, estimated<sup>3</sup> to be 6-10×10<sup>-6</sup> mol per l of cells per h. It has been suggested that the apparently large excess capacity of the Ca2+ pump both gives the required sensitivity to a pump incapable of regulating its Ca2+ affinity4 and ensures against Ca2+ toxicity in certain red cell activities<sup>5</sup>. The results presented here, however, show that circulatory shear stresses enhance the Ca<sup>2+</sup> permeability of the membrane, and suggest that greater demands are imposed on the Ca<sup>2+</sup> pump *in vivo* than had been previously thought. We find that the passive permeability to Ca<sup>2+</sup> is increased by up to an order of magnitude and the Ca2+-stimulated ATPase activity is strongly enhanced when red cells are sheared at physiological rates in a rotational viscometer.

It has long been known that the shape and deformability of erythrocytes can influence blood viscosity<sup>6</sup> and hence, in principle, blood flow in the circulation, but effects of fluid shear on cell membrane reactions have been demonstrated only recently<sup>7-9</sup>. Here, examination of the effect of shear rate on Ca<sup>2</sup> uptake into ATP-depleted and ATP-rich human erythrocytes showed a shear-dependent increase in the uptake of <sup>45</sup>Ca<sup>2+</sup> into the cells (Fig. 1), the uptake rate being constant for at least



Shear rate-dependent 45Ca2+ uptake into human erythrocytes. Blood from a healthy volunteer was obtained by venipuncture with a 20-gauge needle in a 10-ml EDTA-vacutainer and tested the same day. Erythrocytes were separated from plasma by centrifugation at 3,000g for 10 min and washed twice in isotonic saline buffered with 20 mM sodium phosphate, pH 7.4. Packed cells were suspended at a haematocrit of ~10% in a solution containing 150 mM NaCl, 5 mM KCl, 15 mM Tris-HCl, 2 mM MgCl<sub>2</sub>, 5 mM iodoacetic acid, 5 mM adenosine and 0.1 mM EGTA at pH 7.4. Half of the sample was left on ice (ATP-rich) and the other half incubated at 37 °C for 3-4 h (ATP-depleted)<sup>16</sup>. Cells were then washed twice in the medium in which the <sup>45</sup>Ca<sup>2+</sup> was to be measured: 150 mM NaCl, in the medium in which the "Ca" was to be heastiful! 130 mM NaCl, 5 mM KCl, 15 mM Tris-HCl, 2 mM MgCl<sub>2</sub> and 2 mM CaCl<sub>2</sub>. "45 CaCl<sub>2</sub> was added (50–70  $\mu$ l of a 0.5 mCi ml<sup>-1</sup> solution) to 10 ml of the erythrocytes suspended at a haematocrit of 39.5–45%. The suspension was sheared in a variable-speed cone-plate Brookfield model LVT "SynchroLectric" viscometer for 15–30 min at various shear rates at 37 °C. The uptake of Ca<sup>2+</sup> into erythrocytes was assessed by a modification of the method reported by Lew and Ferreira<sup>4</sup>. Aliquots (100 µl) of the cell suspension before and after shearing were layered on 200 µl of Ficoll-Paque solution (Pharmacia) in a 400-μl centrifuge tube and spun for 2 min in a Beckman/Spinco 152 microfuge. The extracellular medium (top layer) and the Ficoli-Pacque solution (middle layer) were removed by aspiration from the packed erythrocytes (bottom layer). A very slight red colour in the Ficoll-Pacque solution was observed occasionally, indicating that minimal haemolysis had occurred at the shear rates tested. The walls of the centrifuge tube were wiped dry with a cotton swab and 100 µl of 10% perchloric acid were added to the tube. The tube was capped, vigorously shaken and the resulting precipitate packed by centrifugation for 1 min in the microfuge. Samples of 100 µl of supernatant were placed in 10 ml scintillation fluid (Amersham) and counted in a Beckman LS-3133P scintillation counter. Calcium uptake was expressed per l of cells per h based on the initial haematocrit values of the cell suspensions, as determined with a Clay-Adams micro-haematocrit centrifuge. The results shown are the means ±s.d. of four (ATP-depleted; O) or three (ATP-rich; •) determinations of cells from volunteer 1.

30 min. Figure 1 shows the increase in the rate of calcium accumulation by red cells depleted of their ATP following 15-30 min shear at various shear rates. Even at the lowest shear rate tested (120 s<sup>-1</sup>) there was a significant increase (P < 0.05) in the Ca<sup>2+</sup> taken up by the ATP-depleted erythrocytes compared with control cells which were only gently mixed. The increase in Ca<sup>2+</sup> associated with the cells was due to uptake and not binding to extracellular sites, as washing the cells in 10 times their volume of isotope-free buffer had no effect on the 45Ca<sup>24</sup> uptake measured and ATP-rich erythrocytes showed a much lower <sup>45</sup>Ca<sup>2+</sup> uptake (Fig. 1). The small <sup>45</sup>Ca<sup>2+</sup> uptake seen in ATP-rich cells may reflect exchange with a pool of Ca2+ inaccessible to the pump rather than net uptake, as about 10-15% of the erythrocyte calcium is present in such a compartment 10.11. However, this fraction of  $^{45}\text{Ca}^{2+}$  also seems to increase slightly with shear rate. We estimate this exchangeable pool of Ca2+ to be 4-5 µmol Ca<sup>2+</sup> per 1 of cells, which agrees with previous reports<sup>11</sup>.

The alternative explanation that the Ca2+ uptake into ATPrich cells represents saturation of the Ca<sup>2+</sup> efflux mechanism seems unlikely because the Ca<sup>2+</sup> pump is capable of restoring Ca<sup>2+</sup> to resting levels within a few minutes at 37°C (ref. 12). Therefore, the most likely explanation for the greater 45Ca2 uptake in ATP-depleted than ATP-rich cells is that the ATP-dependent Ca<sup>2+</sup> pump rapidly removes the Ca<sup>2+</sup> taken up when sufficient ATP is present. This explanation is supported by experiments in which active Ca2+ extrusion from ATP-rich cells

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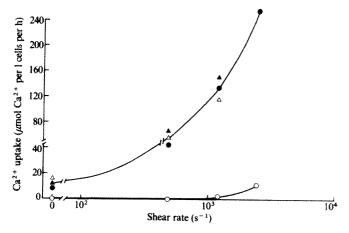


Fig. 2 Effect of La3+ on shear rate-dependent 45Ca2+ uptake into human erythrocytes. The experiments were performed as described in Fig. 1 legend, except that 0.2 mM LaCl<sub>3</sub> was added to the erythrocyte suspension before shearing was begun. A single experiment from volunteer 2 is shown. ATP-rich cells in the absence of La<sup>3+</sup> ( $\bigcirc$ ) and in the presence of 0.2 mM La<sup>3+</sup> ( $\bigcirc$ ); ATP-depleted cells in the absence of La<sup>3+</sup> ( $\triangle$ ) and in the presence of 0.2 mM La3+ (A).

was blocked with La3+ (Fig. 2), which has previously been shown to abolish the active Ca<sup>2+</sup> pump in resealed ghosts<sup>13</sup> and intact cells<sup>14</sup> without blocking the passive Ca<sup>2+</sup> permeability<sup>15</sup>. In the presence of 0.2 mM lanthanum, <sup>45</sup>Ca<sup>2+</sup> uptake showed the same shear dependence in both ATP-rich and depleted cells as found for ATP-depleted cells in the absence of La3+. These results suggest that shear increases the Ca2+ permeability of the erythrocyte membrane through an effect on a La3+-insensitive Ca2+ channel.

While the net accumulation of 45Ca2+ into ATP-rich cells was only weakly shear dependent, Ca2+-dependent ATPase activity in these cells increased strongly with increasing shear rate over the same range as that used in the Ca<sup>2+</sup> uptake experiments (Fig. 3). The experiments were performed in ATP-rich cells treated with 5 mM iodoacetamide to limit glycolysis and the reincorporation of liberated  $P_i$  into  $ATP^{16}$ .  $(Na^+ + K^+)ATP$  and Mg<sup>2+</sup>-ATPase activities were at most only slightly affected (Fig. 3). The Na<sup>+</sup> permeability may thus be much less shear sensitive, or iodoacetamide may have rapidly brought the Na+ concentration in the cells to values saturating the Na<sup>+</sup> pump.

Circulating erythrocytes are subjected to periodic fluctuations in shear stress, conditions being most extreme in the microcirculatory beds. These stresses induce continuous dramatic deformations of the cell membranes. The present results obtained with the physiological shear rate range<sup>6</sup> of 50-

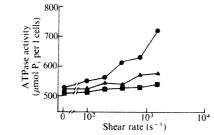


Fig. 3 Effect of shear rate on ATP turnover in ATP-rich human erythrocytes. ATPase activities were measured before and after shearing ATP-rich erythrocytes in the medium used in the <sup>45</sup>Ca<sup>2+</sup> uptake experiments in Fig. 1, but which also contained 5 mM iodoacetamide. The contribution of  $(Ca^{2+}+Mg^{2+})ATPase$  ( ) was measured in the presence of 0.1 mM ouabain;  $Mg^{2+}-ATPase$  ( ) was measured in the absence of  $CaCl_2$  and the presence of 1 mM EGTA and 0.1 mM ouabain;  $(Na^++K^+)ATPase$  ( ) was measured in the absence of  $CaCl_2$ .  $(Ca^{2+}+Mg^{2+})ATPase$  activity was defeate at the arther interest of  $CaCl_2$ . defined as the ouabain-insensitive activity determined in the presence of CaCl<sub>2</sub> minus the ATPase activity determined in the presence of 1 mM EGTA and no Ca<sup>2+</sup>. Samples (200 µl) of erythrocyte suspensions were placed in 1.5-ml centrifuge tubes with 200 µl ice-cold 10% perchloric acid and centrifuged for 1 min in an Eppendorf 5412 centrifuge. Inorganic phosphate was determined in each 200 µl of the supernatant by the method of Fiske and Subba Row22. Results are from a single experiment from volunteer 1.

5,000 s<sup>-1</sup> indicate that, coincident with such deformation, large increases in Ca2+ permeability occur. Hence, the level of internal Ca2+ in erythrocytes could show large transient increases in those parts of the circulation where shear stress is highest. Increased intracellular Ca2+ activity has been shown to increase the suspension viscosity of red cells without significantly affecting cell volume or unstressed shape 17. Ca2+-stimulated dephosphorylation of membrane substrates has also been correlated with the shape and shear resistance of erythrocyte membrane preparations<sup>18</sup>. The relationship between Ca<sup>2+</sup> permeability, Ca2+-dependent ATPase and pump activity, and intracellular biochemical events which are sensitive to Ca2+ levels may therefore be much more dynamic in the circulation than had previously been thought. Viewed in this light, even the twofold deficiencies in Ca<sup>2+</sup> pump activity seen in red cells from cyctic fibrosis patients<sup>19</sup> or in sickle cells<sup>20,21</sup> could acquire greater significance than has hitherto been afforded them.

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## Alisphenoid equivalents in placentals, marsupials, monotremes and fossils

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The interpretation of the wall of the brain case between orbit and ear has important implications for mammalian phylogeny. The fossil mammalian groups morganucodonts, triconodonts and multituberculates possess a reduced alisphenoid and a large anterior process of the petrosal. In this they resemble monotremes. By contrast 'therians' (marsupials, placentals and their putative ancestors) have a large alisphenoid and no anterior process. Palaeontologists reject forms with a large anterior process from a position close to the ancestry of therians, though uncertain of the timing of the divergence1,2. The development of the brain case of monotremes is crucial here. The view that their anterior process is a laminar outgrowth of the petrosal3 has been widely accepted. Recent work has shown that this is not true in echidnas<sup>4,5</sup> and here I confirm the suggestion<sup>6</sup> that it is not true in platypus. The ossification pattern of living mammals indicates the possibility of close affinity between 'therian' and 'nontherian' mammals.

The area of interest develops in the lateral wall of the cavum epiptericum, the cartilaginous ala temporalis in front and the cartilaginous otic capsule (future petrosal) behind. In all mammals a fibrous spheno-obturator membrane<sup>7</sup> lies between these, extending up to the edge of the chondrocranium lateral to the ophthalmic nerve. Terminology of this area has varied. Here I use 'processus ascendens' for the part of the ala between ophthalmic and maxillary nerves; 'lamina ascendens' for the part of the ala between maxillary and mandibular nerves. The English word 'process' signifies an adult osteological feature and 'lamina' is confined to development. I propose here that the more recent term 'lamina obturans'4-6 used for membrane bone developing in the spheno-obturator membrane of monotremes may usefully be extended to the very similar field of membrane bone here in therians. This membrane bone is very variable in its development and fate in Recent mammals and may well be the key to the evolution of this region in advanced therapsids.

I present here two stages from a developmental series of serially-sectioned platypus nestlings. The younger specimen has a small lamina obturans: a single sheet of membrane bone entirely separate from the otic capsule and ala temporalis (Fig. 1a). The older specimen, used by Watson<sup>3</sup>, was the only one available to him having a lamina suitable for histological study. He described it as a forward outgrowth of the petrosal, founding the prevalent morphological view. In this specimen the area of synostosis between the lamina obturans and endochondral bone in the otic capsule is very small (Fig. 1b); in the younger specimen the otic capsule shows no sign of endochondral ossification here. It is clear from these and other specimens that the platypus lamina obturans is an independent centre of intramembranous ossification within the spheno-obturator

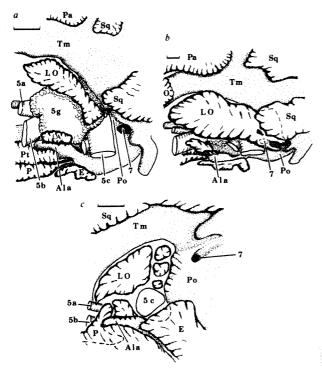


Fig. 1 Temporal regions of a, Ornithorhynchus anatinus, 168 mm snout-tail (ST), lateral aspect; b, O. anatinus, 250 mm ST, lateral aspect; c, Tachyglossus aculeatus, 174 mm ST, ventral aspect. Scale bar, 1 mm. Cartilage is shown stippled, bone with edges scalloped. a Shows a large lamina obturans (LO) barely touching the petrosal (Po). b Has the lamina synostosed with the petrosal in the area overlapped by the squamosal (Sq). c Is drawn from the oldest available specimen: the temporal wing of the palatine and lamina ascendens' (LA) were ossified but the position of other ossifications in the membrane and petrosal are based on refs 4, 5. Ala, ala temporalis; E, ectopterygoid; P, palatine; Pt, pterygoid; Tm, taenia marginalis. 5a,b,c,g, Ophthalmic, maxillary and mandibular nerves and trigeminal ganglion, respectively. 7, Foramen of facial nerve.

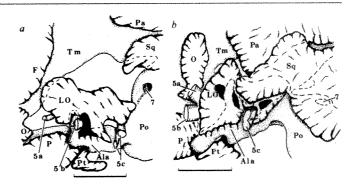


Fig. 2 Temporal regions of a, Didelphis virginiana, 24 mm crown-rump (CR), lateral aspect; b, Erinaceus europaeus, 14.5 mm head length (HL), lateral aspect. Same scale as in Fig. 1. There is an extensive area of membrane bone with similarity to the monotreme lamina obturans in each, but there is little endochondral ossification in the ala temporalis. The dark areas in each lamina indicate schematically where, in younger specimens (14 mm CR opossum, 13 mm HL hedgehog), the earliest ossification in adjacent cartilage. The great variability of the ala temporalis in mammals is illustrated by the presence in the opossum of a cartilaginous processus ascendens between 5a and 5b, while the hedgehog lacks this but has a lamina ascendens between 5b and 5c.

membrane, analogous to the multiple centres described in echidnas  $^5$  (Fig. 1c). Watson  $^3$  lacked younger platypus having this bone and overlooked the possibility of its independent origin.

The structure of this region in monotremes may be summarized as follows: in platypus the small ala temporalis ossifies quite early, with a small perichondrial extension into the spheno-obturator membrane ('lamina ascendens'5). A single lamina obturans appears which fuses first with the petrosal and later with orbitosphenoid, squamosal, palatine and 'lamina ascendens'<sup>5</sup>. Other workers<sup>4,5</sup> have shown that the echidna lamina obturans appears late as several centres; my specimens show here early signs of ossification consistent with these reports, but lack later juvenile stages. The ala temporalis is smaller than that of the platypus but ossifies early and produces a larger perichondrial extension ('lamina ascendens'<sup>5</sup>) into the spheno-obturator membrane, as do the palatine and the anterior margin of the petrosal. In echidnas, closure of the suture between lamina obturans and petrosal may be later than with adjacent elements<sup>5</sup>. It is still visible, though unlabelled, in Watson's figures of echidna (G. T. MacIntyre, personal communication).

All recent therians have a spheno-obturator membrane, in which membrane bone appears earlier than in monotremes, often before endochondral ossification in the ala temporalis, unlike the monotreme 'lamina ascendens'. It appears close to the ala and extends back to suture with the squamosal and tegmen tympani. It is very variable in shape and number of centres. The opossum (Fig. 2a) has a pattern similar to the platypus; the hedgehog (Fig. 2b) has three centres. This membrane bone is histologically and anatomically similar to the lamina obturans of monotremes and forms most of the blade of the alisphenoid of therians, the ala temporalis forming a usually smaller portion from root to nerve exits.

smaller portion from root to nerve exits.

Previous analysis<sup>8-10</sup> holds that the therian alisphenoid, while histologically a complex of membrane and cartilage bone, should be treated morphologically as a derivative of the epipterygoid of primitive tetrapods, the membrane bone being merely a large perichondrial extension of the processus ascendens of the epipterygoid which has been modified and reduced to the ala temporalis. This view is simple and very effective, but note that it was founded before information on the true development of monotremes was available, before excellent

fossil cranial material of Mesozoic mammals was known and at a time when the alisphenoid-epipterygoid theory of synapsid evolution was still controversial<sup>3,11,12</sup>. We now need to take account of the extent of this membrane bone, present in all mammals in a large part of the region, and to allow for its very variable pattern, in taxonomic analysis.

There seems to be no fundamental difference between therians and monotremes in the early development of this bone. Differences arise only when it fuses with its neighbours. It may well have been that the precursors of mammals had membrane bone in this region: cynodonts may have had an anterior part contributing to the broad blade of the epipterygoid and a posterior forming the smaller anterior process of the petrosal. In other groups the proportions may have varied: in triconodonts the anterior process is larger and the epipterygoid blade smaller, while in multituberculates the membrane-bone area may have contributed extensively to the petrosal to the exclusion of the diminutive alisphenoid (epipterygoid). In view of the variable pattern found in living forms and the difficulties of establishing detailed osteological homologies in fossils, it must surely be dangerous to erect two groups, however informally, based on

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the adult pattern of this region. Development shows that there is no 'monotone pattern' and therefore fossils may not be categorized as sharing it.

Excellent skull material of morganucodonts<sup>1</sup>, triconodonts<sup>2</sup> and multituberculates 13,14 is now being studied. It must be appreciated that the blade of the alisphenoid and the anterior process of the petrosal are developmentally very similar and may be morphologically almost equivalent. If Mesozoic, like Recent, mammals formed membrane bone in the area of the lamina obturans, it follows that any form in the fossil record with an expanded epipterygoid, an anterior process of the petrosal, or both, could, by a simple change in the affinity of synostosis during development15, come to possess therian anatomy. Therefore the recognition of the precursor form of therians must rest on other features. The structure of the wall of the cavum epiptericum itself in morganucodonts, triconodonts, multituberculates, cynodonts or even monotremes does not exclude close affinity to therians.

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#### Corrigenda

A correction to the letter '210Pb in surface air at Enewetak and the Asian dust flux to the Pacific' by Karl K. Turekian & J. Kirk Cochran, Nature 292, 522-524.

We have just discovered that the filters sent to us for <sup>210</sup>Pb assay and reported in our recent paper were one-quarter aliquots of the total filter and that the values for volumes transmitted to us subsequently were for the whole samples. This means that Table 1 in our paper must be revised to increase the <sup>210</sup>Pb concentration in Enewetak air by a factor of 4: the correct version is shown below.

Table 1 210Pb and 210Po in air filter samples from Enewetak, April-August 1979

	<sup>216</sup> Pb	<sup>210</sup> Po/ <sup>210</sup> Pb
Date of sampling	$(d.p.m. 10^{-3} m^{-3})$	activity ratio
18 April	$15.3 \pm 2.1$	ener.
26 April	$12.1 \pm 1.0$	$-0.02 \pm 0.08$
3 May	$10.4 \pm 0.76$	$-0.02 \pm 0.03$
10 May	$12.2 \pm 1.0$	$-0.02 \pm 0.03$
15 May	$7.96 \pm 0.72$	$-0.08 \pm 0.03$
20 June	$5.96 \pm 0.20$	$0.14 \pm 0.11$
8 July	$8.00 \pm 0.32$	$0.02 \pm 0.28$
16 July	$3.17 \pm 0.14$	$-0.26 \pm 0.04$
26 July	$3.11 \pm 0.076$	$0.17 \pm 0.07$
4 August	$4.04 \pm 0.16$	$0.05 \pm 0.07$
ver blanks (filter carrie	ed up the sampling tower in ex	(posed conditions)
10 May	$0.424 \pm 0.108$	$-0.09 \pm 0.10$
26 Iuly	$0.180 \pm 0.032$	$-0.05 \pm 0.18$

Sampling date represents the date corresponding to the midpoint of the collec-

This correction changes several relations that we discussed.

- (1) Both the ordinate values for Fig. 1 and the abscissa values in Fig. 2 should be increased by a factor of 4.
- (2) The corrected Enewetak filter results (present Table 1) compare more favourably with previous observations made at Hawaii<sup>2</sup>. Between 15 May and 20 June 1979 at Enewetak, the concentration of <sup>210</sup>Pb was 6-8 d.p.m.  $10^{-3}$  m<sup>-3</sup> and for a similar period (30 May-22 June 1971) at Hawaii the values below the tradegraph. Hawaii, the values below the trade wind inversion were 3.4-19.4 d.p.m.  $10^{-3}$  m<sup>-3</sup> with a mean of 9.3 d.p.m.  $10^{-3}$  m<sup>-3</sup>.
- (3) The regression equation for the calculation of the mean annual concentration of <sup>210</sup>Pb in air becomes

C [d.p.m. <sup>210</sup>Pb 10<sup>-3</sup> m<sup>-3</sup>] = 18.8-85.6×F [d.p.m. <sup>210</sup>Pb cm<sup>-2</sup> yr<sup>-1</sup>]

For a mean flux of  $^{210}\text{Pb}$  of  $0.15\,\text{d.p.m.}$  cm  $^{-2}\,\text{yr}^{-1}$  this yields a mean annual concentration in air of 6 d.p.m.  $^{210}\text{Pb}\,10^{-3}\,\text{m}^{-3}$ .

- (4) The slope (S) of the regression equation correlating  $F_{AI}$  with  $F_{Pb}$  becomes 9.5 µg Al per d.p.m. <sup>210</sup>Pb.  $F_{AI}$  then becomes 0.63 µg Al cm<sup>-2</sup> yr<sup>-1</sup> corresponding to a dust flux (assuming 6.5% Al) of 9.6±5 µg cm<sup>-2</sup> yr<sup>-1</sup>, a closer value to that reported by Duce et al.<sup>3</sup>.
- (5) In Table 2 of our paper the increase by a factor of 4 of the mean Pb concentrations reported there decreases the 'total deposition velocities' by a factor of 4. Thus 'dry' season total deposition velocity becomes  $0.25~{\rm cm~s}^{-1}$  and the 'wet' season becomes  $1.0~{\rm cm~s}^{-1}$ . These values are now lower than our estimated deposition velocities for Hawaii  $(\sim 3 \text{ cm s}^{-1})$  and Bermuda  $(\sim 4 \text{ cm s}^{-1})$ .
- (6) The dust flux calculated for Hawaii using the parameters of the Enewetak <sup>210</sup>Pb-Al regression equation now becomes 93 μg cm<sup>-2</sup> yr<sup>-1</sup>. This is easily in the range of accumulation rates of clay on the deep ocean floor. The conclusion regarding the dominance of the Asian dust flux to the sedimentation in the Pacific remains intact as does the suggestion that post depositional homogenization has occurred.
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In the letter 'Is the similarity of monozygotic twins due to genetic factors alone' by K. Gärtner and E. Baunack, Nature 292, 646-647, in Table 1 mean  $\pm$ s.d. of the MZ should be  $26.41 \pm 2.58$  instead of  $26.2 \pm 2.66$ , and in Tables 1 and 2 the  $s_b^2$  values of 51- and 61-day old DZ should be -1.18 and -2.49 instead of the printed positive values. In Table 2, the calculated F values of the  $s_b^2$  of MZ and DZ are statistically insensitive; therefore the F and P values of  $s_b^2$  should be disregarded.

The title of the letter by Doyle R. Watts and Andrew M. Bramall in Nature 293, 638-641 should read 'Palaeomagnetic evidence for a displaced terrain in Western Antarctica'.

In the letter 'SB: a new HLA-linked human histocompatibility gene defined using HLA-mutant cell lines', Nature 293, 747-749, the present address of Fritz H. Bach should read 'Immunobiology Research Center, University of Minnesota, Minneapolis, Minnesota 55455, USA

In the review article 'A new era in mammalian gene mapping: somatic cell genetics and recombinant DNA methodologies' by F. H. Ruddle, Nature 294, 115-120, on p. 119 ' $\alpha$ -globin' should read ' $\beta$ -globin' throughout. In Table 1, the chromosome positions for the mouse  $\alpha$ -globin pseudogenes are sited incorrectly: pseudogene  $\psi$ 3 maps to chromosome 15 and pseudogene  $\psi 4$  to 17.

#### Presence and physiological role of presynaptic inhibitory α<sub>2</sub>-adrenoreceptors in guinea pig atria

IT was concluded by Angus and Korner<sup>1</sup> "presynaptic  $\alpha$ -adrenoreceptor that modulation by synaptically released noradrenaline plays no part in cardiac sympathetic transmission".

Evaluation of their results and experimental methodology suggests that this criticism may be premature because there are important methodological differences between this study and several others which showed evidence for presynaptic α-adrenoreceptor modulation of cardiac neurotransmission. As the consensus of opinion supports a physiological role for presynaptic  $\alpha$ -adrenoreceptors in the heart, it is useful to identify and examine these experimental differences in more detail.

(1) The study was carried out with guinea pig right atria in Krebs' solution which contains 1.2 mM Mg<sup>2+</sup>. In these experimental conditions the resting rate<sup>1</sup> is only 110 beats per min while in most articles dealing with spontaneously beating guinea pig atria and using Locke's solution, which does not contain Mg<sup>2+</sup>, the resting rate<sup>2-5</sup> is between 200 and 230 beats per min.

Angus and Korner state that "phentolamine 0.3-10 µM had no significant effect on the resting atrial rate or the dose-response curve to noradrenaline"1. The fact that their resting rate was already very low may explain their findings.

However, the authors1, without admitting their different resting atrial rate, presented these results as conflicting with findings by Langer et al.6 of a negative chronotropic effect and attenuated chronotropic responses to exogenous noradrenaline in the presence of 31 µM phentolamine. However, the results of Langer et al.6 were obtained with Locke's solution in conditions in which the resting atrial rate<sup>6</sup> was 210 beats per min. When repeating their experiments with Locke's solution Angus and Korner found that, as reported previously6, even 10 µM phentolamine produced a significant negative chronotropic effect in guinea pig atria (J. A. Angus, personal communication). Angus and Korner did not determine phentolamine  $31 \mu M$ whether antagonized responses of atria to exogenous noradrenaline as reported by Langer et al.6.

(2) Instead of accelerans nerve stimulation<sup>6</sup> the experiment by Angus and Korner used field stimulation<sup>1</sup>; clearly, the latter is a less physiological stimulus than nerve stimulation, and this difference in methodology may be one of the reasons

for their negative results. In support of the results of Langer et al.6 it was recently reported that both phentolamine and produce a significant yohimbine enhancement of the inotropic responses to sympathetic stimulation in guinea pig left atria7.

(3) Potentiation of the chronotropic responses to accelerans nerve stimulation at 0.5 Hz was reported in the presence of 0.1 µM phentolamine6. Using one or four pulses of field stimulation applied during the refractory period, exposure to 10 µM phentolamine failed to potentiate the chronotropic responses to field stimulation1. Recent experiments in which field stimulation was applied in guinea pig atria using four pulses at 2 Hz showed that exposure to 3 µM phentolamine did not significantly affect the overflow of <sup>3</sup>Hnoradrenaline8, thus confirming the observation of Angus and Korner<sup>1</sup>. However, when field stimulation was applied at 2 Hz using a total of 16 pulses, to 3 µM phentolamine exposure enhanced the overflow of the labelled transmitter as well as the peak chronostimulation8. responses to Obviously, the negative feedback regulation of noradrenaline release mediated by presynaptic  $\alpha_2$ -adrenoreceptors depends on the frequency and duration of neuronal activity. Failure to observe potentiation of the positive chronotropic responses to field stimulation in the report by Angus and Korner<sup>1</sup> is clearly related to the fact that they used too short a period of stimulation. These results8 confirm the findings of Langer et al.6 obtained with accelerans nerve stimulation.

It is premature to reach definitive conclusions, as did Angus and Korner, about the absence of feedback modulation of noradrenaline release based on negative results originating from inadequate experimental conditions which are restricted to very brief periods of stimulation. If these authors had explored a wide variety of frequencies of stimulation using different durations of the period of stimulation, their conclusions would have been quite the opposite, as clearly demonstrated by Story et al.8.

(4) Although the presence of presynaptic inhibitory  $\alpha_2$ -adrenoreceptors in guinea pig atria is questioned by Angus have and Korner<sup>1</sup>, thev shown subsequently that clonidine, an  $\alpha_2$ adrenoreceptor agonist, decreases the responses to field stimulation in this tissue9. In addition, clonidine reduces the inotropic response to sympathetic nerve stimulation in guinea pig left atria and this effect is antagonized by 5 µM phentolamine or 0.1 µM yohimbine (ref. 7). These results clearly support the view that presynaptic inhibitory  $\alpha_2$ -adrenoreceptors are present in noradrenergic nerve endings of the peripheral nervous system 10-12. These release-modulating presynaptic  $\alpha_2$ -adrenoreceptors are definitely of pharmacological relevance in both in vitro and in vivo conditions 13,14 The possible physiological role of presy- $\alpha_2$ -adrenoreceptors in naptic noradrenergic neurotransmission is supported by the findings that  $\alpha_2$ adrenoreceptor blocking agents enhance both the electrically evoked release of noradrenaline and the end-organ responses to sympathetic nerve stimulation 10-12 provided suitable experimental conditions are used.

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ANGUS AND KORNER REPLY-In our paper1 we tried to establish whether or not neurally released noradrenaline could activate an auto-inhibitory feedback on the sympathetic nerve terminals of the guinea pig right atrium and concluded that it did not. There were differences between our methodology and those of Langer and co-workers and we are grateful for the opportunity of discussing these.

(1) Bath solution. We used a standard bicarbonate-buffered solution for heart muscle<sup>2-4</sup> of pH  $7.58 \pm 0.01$  (n = 5) bubbled with 95% O<sub>2</sub>, 5% CO<sub>2</sub>, compared with Langer and co-authors<sup>5-7</sup> who routinely use Mg<sup>2+</sup>-free Locke's solution bubbled with 100% O<sub>2</sub>. We found that this Locke's solution has a pH of 8.19 ± 0.06 (n=4), a similar finding to Trendelenburg8 and well outside the normal physiological range. The higher resting rate in Locke's solution of 180-210 beats per min compared with our rate of 110-150 beats per min are accounted for by the well known effect of pH on resting atrial rate<sup>9,10</sup> rather than the lack of Mg<sup>2+</sup> which, parenthetically, is present in guinea pig

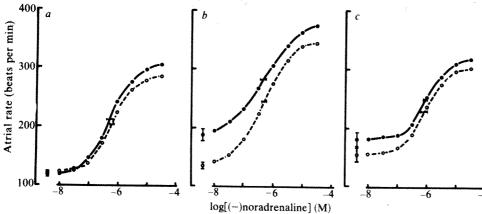


Fig. 1 Effect of 10 µM phentolamine (30 min contact) on resting atrial rate and on chronotropic responses to exogenous (-)noradrenaline in isolated guinea pig right atria in three different bath condi tions. a, Krebs' solution, pH 7.58, temperature 37 °C (n = 4): control, first curve; phentolamine, fifth curve (data from ref. 1). b, Krebs' solution, pH 7.58, temperature 38.4 °C (n = 6); control, first curve; phentolamine, second curve. c, Locke's solution, pH 8.19, temperature 37 °C (n = 4); control, first curve; phentolamine, second curve. Solid symbols = control; open symbols = phentolamine. Means ± s.e.m. at the left of each graph are the resting atrial rates before the start of the noradrenaline curve.

plasma  $(0.86 \pm 0.08 \text{ mM}, n = 4)$ . As pointed out by Blinks and Koch-Weser<sup>11</sup>, "the solution that produces the most vigorous contractions is not necessarily the most physiological one... The most physiological solution is one that most closely the normal extracellular resembles fluid . . .

(2) Phentolamine. We agree that the lower resting rates in our preparations1 may have masked the negative chronotropic effect of high phentolamine concentrations (10 µM). By raising the temperature of the bath from 37 °C to 38.4 °C (body temperature in conscious guinea pigs) the resting rates were now  $186 \pm 29$  (n = 6), significantly higher than at 37 °C. In these conditions, as with Locke's solution at 37 °C, 10 µM phentolamine significantly lowered the resting rate (Fig. 1). However, the sensitivity (threshold and ED<sub>50</sub> value) and range (change in rate) of the exogenous noradrenaline concentration-response curves were not significantly altered by phentolamine (negative log molar ED<sub>50</sub> values: Krebs', 38.4 °C (n = 6), control = 6.33 ± 0.11, phentolamine 6.31 ± 0.09; Locke's 37 °C (n = 4), control = 6.03 ± 0.03, phentolamine  $6.08 \pm 0.09$ ). Therefore, we cannot conclude that 10 µM phentolamine antagonizes the atrial responses to noradrenaline, a suggestion used by Langer and co-authors to explain why phentolamine (3.1 µM) failed to potentiate atrial responses to nerve stimulation on two occasions<sup>5,6</sup> despite a large but variable increase in 3Hnoradrenaline release. It is interesting that Langer<sup>5</sup> has observed an increase in the rate response after 0.31 µM phentolamine with nerve stimulation at 0.5 Hz for 10 s, but that in the same study<sup>5</sup> the supporting evidence of increased noradrenaline release was made at nerve stimulation of 4 Hz for 60 s, a stimulus that produces near maximum rate response<sup>6</sup>. Higher concentrations of phentolamine (31 µM) appeared depress the resting atrial rate and to shift the exogenous noradrenaline concentration-response curve to the right<sup>5</sup>. The relevance of these data is not clear, as Langer and colleagues<sup>5</sup> presented no results on 3H-noradrenaline release or rate response at this concentration.

(3) Accelerans nerve stimulation. The advantage of field stimulation is that all nerve varicosities are depolarized synchronously by each field pulse<sup>12</sup>. With nerve stimulation there is some evidence 13,14 that action potentials may not always invade all the terminal varicosities. Stjärne<sup>15</sup> points out that drugs may potentiate noradrenaline release by recruitment of varicosities. Therefore, to test the hypothesis of auto-inhibitory feedback, it is logical to eliminate varicosity recruitment by using field stimulation, a technique used by others in examining noradrenaline release atria4.

Therefore, we believe that the methodological points raised by Langer do not invalidate our conclusion that physiologically released noradrenaline does not activate auto-inhibitory receptors in the conditions of our assay. If the widely championed view that presynaptic  $\alpha$ -adrenoreceptors are important in sympathetic transmission in physiological conditions, we assert that phentolamine should have raised responses after four pulses. After all, a "pulse-to-pulse modulation of noradrenaline release" was proposed by Rand and colleagues 16 and has often been quoted by Langer<sup>17</sup>.

Recently, as Langer points out, Story and colleagues have confirmed our results that the tachycardia resulting from four field pulses that give up to 50% maximum response is unaffected by phentolamine. However, an increase in response was observed in the presence of phentolamine after 16 field pulses, which cause near maximal tissue response. In our experiments, field pulses were delivered only in the atrial refractory period to avoid arrhythmia, in marked contrast to the random delivery in experiments by Story et al. 18. They have now described conditions in which the feedback loop seems to operate even for four field pulses provided the frequency is ≤1 Hz. If the conditions for the operation of the feedback loop are so critical, it is difficult to imagine how a nerve varicosity can be involved in feedback control in normal conditions when not every impulse from nerve trunk stimulation invades each varicosity.

We too have found that clonidine inhibits the sympathetically mediated

tachycardia and that this action is antagonized by low concentrations of phentolamine  $(0.1 \mu M)^{19}$ . Therefore, we agree that there is an important pharmacological target on cardiac sympathetic nerve terminals which is probably related to a presynaptic  $\alpha$ -adrenoreceptor and where some imidazolines show high affinity.

Finally, our paper has, of course, not examined all conditions of stimulation because we attempted to examine the role of presynaptic  $\alpha$ -adrenoreceptors in conditions producing a moderate tissue response. Clearly, our paper and that of Story et al. highlight the need for caution in assuming a general operation of autoinhibitory feedback in cardiac sympathetic transmission.

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# Satellite-sensed turbulent ocean structure

RECENTLY Gower et al.1 have reported results concerning interesting concentration of phytoplankton measured by the Landsat multispectral scanner on 19 June 1976 in a region south of Iceland. The observed concentration fluctuation spectra is found to follow approximately a  $k^{-2.92}$  law, close to the  $k^{-3}$  law proposed by Charney<sup>2</sup> for the energy spectrum of quasi-geostrophic flows. They conclude that this result is consistent with the hypothesis that phytoplankton behaves like a passive scalar convected by ocean currents.

The quasi-geostrophic theory of Charney<sup>2</sup> predicts  $k^{-3}$  laws for both horizontal kinetic energy and temperature variance, however, temperature is not a passive scalar, but the vertical derivative of the horizontal stream function. It is the constraints of total (kinetic plus available potential) energy conservation potential enstrophy conservation

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle\psi_x^2+\psi_y^2+\psi_z^2\rangle=0$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle\psi_{xx}^2+\psi_{yy}^2+\psi_{zz}^2\rangle=0$$

together with the assumption of statistical isotropy, which yields for any of the derivatives  $\psi_x$ ,  $\psi_y$ ,  $\psi_z$  a power spectrum following a  $k^{-3}$  law in the potential enstrophy cascading range.

Now, if we consider a passive scalar convected by the quasi-geostrophic motion, as we may expect phytoplankton is, the situation must be quite different. For simplicity we neglect baroclinic effects: for pure two-dimensional turbulence, it can be shown<sup>3</sup> using either phenomenological arguments or statistical closures, that the spectrum of a passive scalar is proportional to the spectrum of the quantity which cascades. Therefore in the enstrophy cascading inertial range, the spectrum of a passive scalar should follow a  $k^{-1}$  law, like the enstrophy (and not the energy) spectrum. There seems to be no reason why this result could not be extended to quasi-geostrophic turbulence which follows a similar phenomenology. A  $k^{-3}$  energy spectrum for the ocean currents would then imply a  $k^{-1}$  law for the passive scalar; conversely, a  $k^{-3}$  spectrum for the passive scalar variance of the type reported by Gower et al.1 would imply a much steeper slope  $(k^{-5})$  for the current kinetic energy.

Such steep slopes have been found in fact in numerical simulations of forced two-dimensional turbulence4; the consequent discrepancy with the Kolmo-

gorov-Kraichnan-Leith theory<sup>5</sup> which predicts a  $k^{-3}$  law is still an open question. For instance, the presence of intermittency effects would yield a steepening of the energy spectrum4; but still, neither kinetic energy spectra nor temperature variance spectra steeper than  $k^{-3}$  have yet been observed experimentally.

The quasi-geostrophic theory of Charney2 neglects the influence of upper and lower boundaries; it is therefore likely to be valid in the deep ocean rather than near the surface. Near the surface the dynamics is likely to involve the occurrence of frontal systems, and one should refer to the quasi-geostrophic theory of Blumen<sup>6</sup> rather than to that of Charney2. In Blumen's<sup>6</sup> theory potential vorticity is assumed to vanish

$$\psi_{xx} + \psi_{yy} + \psi_{zz} \equiv 0$$

inside the fluid, and the two invariants are the total energy of the fluid, and the available potential energy at the surface, which both reduce to integrals taken over the surface only:

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle\psi_z^2\rangle=0$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle\psi_{zz}^2\rangle=0$$

The quantity which cascades to smaller scales is the available potential energy at the surface, which therefore exhibits a  $k^{-5/3}$  law characteristic of frontal systems. If we follow this theory, which looks more appropriate than the first one in the case of surface dynamics, we end up with a  $k^{-5/3}$ law of the passive scalar variance. This is again quite far from what has been observed for phytoplankton, although again the presence of intermittency could yield a steeper slope.

It seems, therefore, difficult to infer anything conclusive from the measurements reported by Gower et al.1 concerning a possible behaviour of phytoplankton as a passive scalar. Also, some caution

# **Matters Arising**

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in Nature. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

should be observed regarding regression estimates for spectral slopes. The concept of an inertial range is only asymptotically valid, and the data used by Gower et al. may be close to the internal radius of deformation to yield any sensible approximation to the actual behaviour of the inertial range. Apparently a regression calculated on the range 1-10 km would vield much lower values of the slope.

A secondary point concerns the assumptions in Gower et al.1 that radiance upwelling is a linear function of phytoplankton concentration. The only assumption needed to investigate the passive scalar character of phytoplankton is that radiance upwelling is a function of phytoplankton concentration because any function of a convected quantity is a convected quantity, exhibiting therefore the same spectral behaviour.

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GOWER ET AL. REPLY-In our recent paper<sup>1</sup>, the -2.92 slope found for the spectrum of near-surface phytoplankton variance is not consistent with theories of a passive scalar in a quasi-geostrophic turbulent fluid. The reasoning is based on a subsequent paper by Lesieur et al.2

However, we do not feel that a further search for physical explanations for our spectrum is warranted: phytoplankton are not always a conserved passive scalar. Rather, as microscopic plants in the sea, they grow, at times at rates capable of doubling their weight each day; and they die or are eaten, often at rates comparable with their growth rates. These time scales are short compared with typical time scales for quasi-geostrophic eddies (10 days). Even in the absence of turbulent motion, biological processes can result in similar negative power law spectra<sup>3</sup>. Still it is feasible that areas of abundant phytoplankton may correlate with fluctuations of a dynamic variable, such as upwards

velocity or temperature, in which case the spectra of temperature and phytoplankton variance might be similar.

The point that all convected quantities in a quasi-geostrophic turbulent field will have the same variance spectrum even if they are related in a non-linear manner, if it is correct, increases our confidence that our published spectra are an accurate representation of the near surface phytoplankton variability. We can still envision that constraints of calculating a spectrum, such as the apparent diffusion caused by the finite size of picture elements and the necessity to average over picture elements, might cause convected quantities related in a non-linear manner to have different calculated spectra.

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# Problem of light piping in immunofluorescence studies

In providing evidence from indirect immunofluorescence for the presence of myosin in the stereocilia of cochlear hair cells, Macartney et al.1 present a figure in which fluorescent light, in the pattern of stereocilia, emanates from the apical surface of hair cells. As the arrangement of the actin filaments which form the core of the stereocilium places structural constraints on the location of significant amounts of other proteins, if myosin is present in the stereocilia, it would have to form either the inter-filament crossbridge or be located in the space between the core and the membrane, both of which seem unlikely. Therefore the results of Macartney et al. were puzzling. This led me to consider the possibility that they were an artefact of light piping. I have subsequently learned that Macartney et al. (personal communication) have taken transverse sections of the organ of Corti. showing fluorescence along the lengths of the stereocilia, thus making light piping a less likely explanation of their results. Nevertheless, for the sake of future studies on the localization of immunofluorescence in hair cells we now explain the problem of light piping so that precautions can be taken to exclude it as a source of spurious results.

Light piping was first observed with respect to hair cells in the last century, and

has been described more recently by Kohlloffel<sup>3</sup>. Hair cells, because they have a higher refractive index than the surrounding extracellular medium, behave as optical waveguides (the 'fibre optic effect); light entering these cells obliquely Kohlloffel's treatment of this phenomenon<sup>3</sup> was restricted to the optical phenomenon<sup>3</sup> was restricted to the optical properties of the hair-cell body (which is roughly cylindrical in shape), but other observations suggest that the stereocilia, emerging from the apical surface of the cell, can enhance this effect.

It would be convenient if one could make a detailed description of the exact optical properties of the stereocilia, but this is almost impossible, due to the necessity of solving Maxwell's equations for discrete electromagnetic modes within a complicated geometry (each stereocilium has a diameter of the order of the wavelength of light). Nevertheless, observations of the stereocilium structure<sup>4</sup> enable one to make a rough estimate of the parameters involved in this fibre optic effect. The density of actin alone in the stereocilia is 0.4 g cm<sup>-3</sup>. If we include the contribution of a cross-bridging protein, a reasonable estimate for the total protein density might be  $0.55\,\mathrm{g\,cm^{-3}}$  in the stereocilium core. Using a specific refractive index increment of 0.18 for proteins, one finds a refractive index within the sterocilium of 1.43, as opposed to 1.33 for the surrounding endolymph (assumed to be that of water). Thus, the numerical aperture (NA) for this waveguide, which is a measure of the angular acceptance of the fibre, would be  $\sim 0.5$ , a value quite similar to that which is obtained for the rod outer segments of the retina, a known biological fibre optic structure. The larger the NA, the greater the light-gathering properties of a fibre. This NA value might be compared with that of =0.3 given by Kohlloffel for the body of the hair cell. Although the fibre characteristic term, R, for the stereocilium is quite small (1-3, as opposed to 10-20 for the hair-cell body) and the number of propagating modes will therefore be small for an isolated stereocilium<sup>5</sup>, it is quite probable that collective behaviour of the approximately 100 closely spaced stereocilia on each hair cell will be in the rule. Thus, the stereocilia bundle would still be observed macroscopically to behave as a light pipe.

This effect might be observed in several different ways. One would be to illuminate the cell externally, as Kohllofel did, and look for localized brightness at the stereocilia tips. Alternatively, one might introduce a fluorophore which could freely diffuse within the cell. Light being selectively emitted from the stereocilia tips would then indicate the existence of the fibre optic effect described above. The latter approach already seems to have been tried for reasons other than verifying the existence of this effect. Goldstein

diffused fluorescein diacetate (FDA). which is non-fluorescent and non-polar (lipid soluble), through the membrane of hair cells. The FDA is hydrolysed to fluorescein by nonspecific esterases within the cells. Fluorescein, which is fluorescent, is also polar, so it will not freely diffuse out through an intact membrane. A cell with a completely non-permeable membrane would then show fluorescence increasing with time as more and more FDA diffused in. Permeable cells would lose fluorescence as the rate of fluorescein diffusing out exceeded the rate of FDA diffusing in. The object of Goldstein's study was to determine changes in the permeability of hair-cell membranes as a function of sound exposure and oxygen deprivation. However, he observed a particular anomaly described as "spots of fluorescence at the top of each cell... Because of the 'W' pattern of these dots, these presumably were the cilia of the hair cells". Because fluorescein does not bind to any known cellular structures, and because the light-piping effect was not considered, Goldstein assumed that the stereocilia are separated from the hair-cell cytoplasm by a unique membrane of their own. This postulated membrane would allow fluorescein to be retained within the stereocilia, causing the localized fluorescence. As no such membrane structure has ever been observed in detailed electron microscopic investigations, it is more probable that Goldstein's pictures are a good example of stereocilia light piping.

The importance of these points is underlined by the number of people using immunofluorescent localization of proteins in general within biological systems, and particularly those using this technique on hair cells. When one considers the striking resemblance of the photograph published by Macartney et al.1 to those taken by both Kohlloffel<sup>3</sup> (with external illumination) and Goldstein<sup>6</sup> (with diffuse internal illumination), it is obvious that one cannot distinguish between the different sources of the illumination in these photographs. All light seems to be emerging from either the apical surface of the cells or the stereocilia in all three cases.

**EDWARD EGELMAN** 

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# **BOOK REVIEWS**

# The order of life — towards a comparative biology

Ian R. Ball

WERNER Heisenberg once said that a revolution in science could be recognized by old terms acquiring new meanings and by an increase in philosophizing by its practicians. Systematic biology is clearly in the midst of revolution and this is nowhere more clearly indicated than in these two splendid books. Their effect may be long in the making, however, for the shadow of Darwin has indeed fallen colossus-like over systematic biology for over a century and our concepts of biological order in space and in time are suffused with his ideas.

For Darwin, geographical distribution was an indispensable key to nature and he established what has become the traditional approach to this subject. He believed all the leading facts of geographical distribution to be explicable in terms of single centres of creation with subsequent migrations, largely due to chance dispersal, further modification and and multiplication of new forms. In the hands of his followers such as Matthew, Simpson, Darlington and Mayr, biogeography thus became concerned with centres of origin, means and routes of dispersal, climate and the evolution of dominance, and the analysis of biotic components in terms of colonizer elements. Despite the considerable achievements of these authors, their derogation being one of the least attractive aspects of both of these books, their biogeography can now be seen to be incomplete for the Herculean, often polemical, labours of Léon Croizat to combat these ideas are becoming increasingly well known. Arguing that the distributions of a wide variety of organisms over the face of the Earth were not random, but orderly, Croizat stressed ad nauseam that dispersal was no explanation. Recognizing that Earth and life evolve together, he pointed the way towards a synthesis of the geological and biological sciences.

For Croizat the history of life involves periods of mobilism, when species movement occurs in the absence of barriers to such movement, and periods of immobilism, when speciation occurs as the result of the erection of barriers and the fragmentation of ranges. This combination of geological-climatological and allopatric speciation events embraces the concept of vicariance. Thus we have a vicariance biogeography in which successive divisions of a parental biota replace the multiple and successive arrivals or sequential migrations of individual species. Sympatric distributions of closely

Vicariance Biogeography: A Critique. Edited by G. Nelson and D.E. Rosen. Pp.593. ISBN 0-231-04808-4. (Columbia University Press: 1981.) \$45.40. Systematics and Biogeography: Cladistics and Vicariance. By G. Nelson and N. Platnick. Pp.567. ISBN 0-231-04574-3. (Columbia University Press: 1981.) \$45.40.

related species are the primary evidence that dispersal has occurred.

Vicariance Biogeography contains the proceedings of a symposium held at the American Museum of Natural History in 1979, and on the evidence of the published version I am sorry that I missed it. Like most symposium volumes it is diverse, wide ranging and it contains articles covering the full spectrum from pro- to contravicariance biogeography and its relationship to systematics. Brundin gives an especially valuable outline of Croizat's "panbiogeography" but it would be unfair to single out special praise in a volume of uniformly high quality which deals with almost all aspects of biogeography vicariance, dispersal, speciation, plate tectonics, the testability of biogeographical theories and so on. An especially happy idea was that of allowing space for the discussions of each paper and then giving the author the right of reply, and frequently the more interesting points are made in these sections. The breadth and quality of this volume make it indispensable to biogeographers.

The relationship between systematics and biogeography is a recurring issue in both of these volumes. Croizat himself had little interest in the theory of systematics and it was only with the rise of phylogenetic systematics, now transformed to cladistics, that vicariance biogeography came into its own. The importance of phylogenetic analysis to biogeographical enquiry is now generally accepted.

The culmination, or reductio ad absurdum depending on one's viewpoint, of syntheses between cladistics and biogeography is provided by the book written by Nelson and Platnick. Both of these authors, individually and as a set, have written prolifically on the theory of systematics and biogeography, and it is therefore invaluable that they should have consolidated their views into a single testament. Systematics and Biogeography is divided into three unequal parts of uneven value - Form (systematics), Time (ontogeny, phylogeny, palaeontology) and Space (biogeography) - and is concerned with the theoretical structure of

comparative biology and the nature of our knowledge of organismic diversity. In a work laying much stress on methods, it is perhaps ironical that I find myself in agreement with its main conclusions while finding the philosophical and logical arguments leading to these conclusions naive.

In its treatment of biogeography we are taken beyond classical vicariance and the multiple sister-group approach of Hennig and of Brundin to a detailed analysis of organism and area cladograms, their comparison and congruity. The rigorous methods of comparing and summating cladograms, branching diagrams of relationship, yield a truly novel approach whose validity I would not question within the domain of its applicability, which concerns areas of endemism and their interrelationships. But species-level disjunctions, active or passive dispersal, extinction and non-allopatric speciation are all part of the history of life and cannot be legislated out of biogeography. Yet I look forward to the new rigour and energy that will surely be stimulated in comparative biology by this book.

Because of the importance of cladograms, the theoretical arguments behind the systematic sections of Systematics and Biogeography achieve a special significance. Here I find much with which to disagree. These sections are a detailed exposition of a particular or, some will say, narrow point of view. Impunitum numquam beneficium is the motto heading the book, but the authors need not fear that they will lack critical comment. Quieta movere magna merces videbatur may well prove to be their Sallustian refuge.

As a theoretical treatise the book is much concerned with the notions of order, existence and of relationship. The core of the approach, component analysis, involves the contention that cladograms are sets of phyletic trees only one of which is topologically equivalent to the cladogram, and it is cladograms, divorced of evolutionary implications, which have the greater generality and testability (propositions which I would deny) and which thus form the basis of systematic analysis.

Much of the early part of the book concerned with the justification cladograms as assessments of the order nature, an order which presupposes the existence of some sets (taxa) and denial others. But if we abandon the theoretic expectancy of a particular hierarchic order — that is, the evolutional

background - then it must be acknowledged that in strict logic a system has all the orders of which it is capable, and that which is most familiar, or most immediately perceived, may not be the order. It was Kant's problem as to what justifies us in seeing nature as a whole that assumes the form of a logical system and its solution requires theoretical input concerning causality and not mere assessment of pattern. Cladistics as a formal system is a sterile enterprise; it becomes alive only when it deals in terms of generation and transformation, either as a result of human agency (for example the study of languages or of historical texts) or as a result of processes of nature (evolution). It follows that I find the authors definitions of cladograms as synapomorphy schemes lacking such connotations less than useful, their explicit defence of this position notwithstanding. Others will decide for themselves.

There is also a great deal of set theory in the book, but of a simplistic and misleading sort. If one rejects nominalism, and accepts that sets over which quantification is possible exist, then one cannot arbitrarily deny some and not others. If quantification were not possible over all sorts of (admittedly overlapping) biological sets, then comparative physiology, ecology and so forth would not be possible. Logically, the meiofauna is as valid a set as is Aves. The special sense of existence for the hierarchical nonoverlapping sets representing the higher taxa of biological systematics is meaningful only when causal principles of their origins are involved.

What can one make of discussions of sets that are "synonymous"? Does this mean identicality, proper inclusion? This is not always made clear, and some set definitions given in terms of homologous character states may well prove to violate the "vicious circle principle". Concepts of intensional and extensional definition, denotation and connotation, although not explicitly discussed are often confused, and we could have hoped for more profound discussions of "natural" in the sense of "natural classifications", "natural taxa" and "natural kinds". The authors seem unaware that there is a vast philosophical literature dealing with such concepts and, it seems to me, fail to appreciate that these terms have meanings more precise than those of everyday use. We are told that homology does not exist independent of synapomorphy (a clear case of putting the cart before the horse!) any more than a set exists independent of its members, yet most logicians require the null or empty set. I am tware that logicians such as Goodman efer to wholes rather than sets, preferring o do without the null set, but there is no Uttence that Nelson and Platnick have taken this particular route.

ken this particular route.

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because the relevant sections are a very important part of the work. And because they are so lucidly written, despite some lapses into condescension, these sections will have an immediate appeal to those biologists unused to logic and philosophy. Yet they must be read critically, for if it is indeed correct to accept the main conclusions of the book while denying the argument then the philosophy and logic must be re-worked. Nevertheless, I recognize the book as a tour de force of systematic biology; in particular, the synthetic parts such as those linking

systematics and biogeography into one grand structure are masterly. And even if in the historical sections scant attention has been made to my heroes, Kant and Goethe—whose notions of natural order are surely relevant to this book—I would still willingly sing with the angels from Faust: "Wer immer strebend sich bemüht, den können wir erlösen".

Ian R. Ball is Professor in Systematic and Geographical Zoology in the University of Amsterdam, The Netherlands.

# Fresh interpretation of the galaxies

Barry F. Madore

A Revised Shapley-Ames Catalog of Bright Galaxies. By Allan Sandage and G.A. Tammann. Pp.157. ISBN 0-87279-652-3. (Carnegie Institution of Washington: 1981.) \$29.

SEVERAL years ago I walked into the refrigerated plate-storage room of the Dupont telescope at the Las Campanas Observatory in Chile, only to be confronted with a yellow wall of Kodak plate boxes carrying the message "Do not use—Reserved for Allan Sandage". These were the plates that were yet to be exposed to the black skies over the Chilean Andes, and which were destined to complete A Revised Shapley-Ames Catalog of Bright Galaxies.

Nearly half a century ago, Harlow Shapley and Adelaide Ames published their now-famous list of some 1,200 galaxies. Although this was only a small subset of the galaxies observed visually by the Herschels (data from which was compiled by Dreyer in the 1888 edition of the New General Catalogue), the Shapley-Ames Catalog bore the distinction of being quantified in a dimension that transcended the NGC and set the stage for subsequent discussions of the spatial distribution and intrinsic properties of galaxies. The original Catalog was, with a fair degree of success, a complete sample of the apparently brightest galaxies to a magnitude limit of about  $m_{pg} \sim 13$ . The publication of a revised version by Sandage and Tammann is both a natural extension of this work and a bold expression of new convictions.

A Revised Shapley-Ames Catalog is not simply a listing of positions, magnitudes and classifications for the brightest galaxies, it is a personal statement about galaxies and the type of Universe they inhabit. The new Catalog contains much interpreted data, and in Sandage's own words "This Catalog is going to be controversial". Certainly, most of the controversy will arise from the distances that are hidden away in the tabulated

absolute magnitudes of Column 16. And almost as certainly we shall soon see a third edition of the *Reference Catalogue of Bright Galaxies* by the de Vaucouleurs, who, together with Corwin, aimed at providing a different interpretation of the data. The time and energy needed to follow the tortuous paths of logic encountered in determining the distances to galaxies can only leave lesser mortals following on in awe. So there is and will continue to be a widely accepted need for this type of quantitative speculation.

Still, for those wishing to strike out on their own the revised Catalog will provide a wealth of raw data. Radial velocities for all but a handful of galaxies are now available; a comprehensive reference list to the original data is graciously provided; and excellent photographs illustrate the classification system employed.

As dry as a catalogue might potentially be, there is a sprinkling of humour in the text. For example, in Table 10, describing the codes for the sources of plate material used in the galaxy typing, the Uppsala Schmidt is listed as having been used for a total of zero galaxies. One might wonder how many other telescopes should have also been included here.

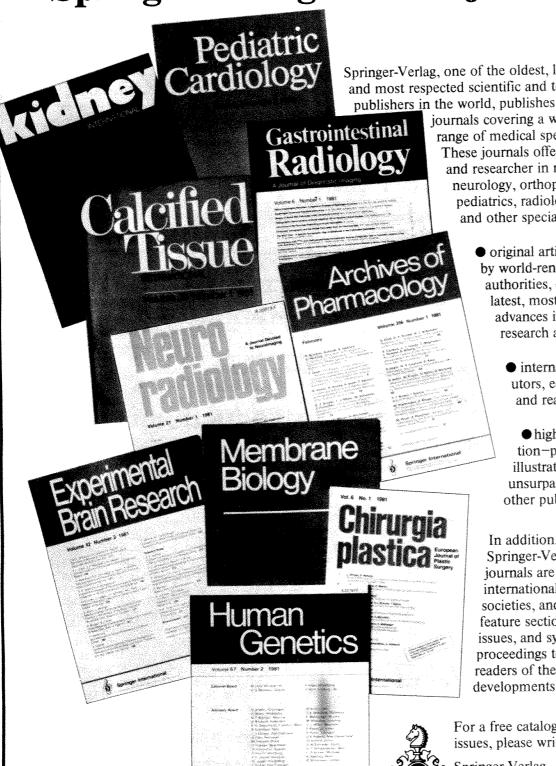
Finally, it is of interest, to me in particular, that "at least 98 per cent of the catalog galaxies can be fitted into the revised Hubble system" while "descriptive terms, such as 'pec' (peculiar), 'disrupted', 'tidal', 'ring', 'jet' and 'edge on' have been added" only in several cases. Those who see the perfection and uniformity of nature give a balance to those who study the pathology.

As for controversy, this is surely a sign of health in an active science. A Revised Shapley-Ames Catalog will be a valuable and welcome addition to the continuing search for answers in extragalactic astronomy.

Barry F. Madore is a Professor in the Department of Astronomy at the University of Toronto.

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# Epidemiology of the townsman's blight

D.G.T. Williams

Rural Conservation in Inter-War Britain. By John Sheail. Pp.272. ISBN 0-19-8232-36-5. (Oxford University Press: 1981.) £16.50, \$49.50. The Countryside: Planning and Change. By Mark Blacksell and Andrew W. Gilg. Pp.262. Hbk ISBN 0-04-711008-2; pbk ISBN 0-04-711009-0. (George Allen & Unwin: 1981.) Hbk £13, \$35; pbk £6.95, \$17.95.

A "widespread general concern" about the scale and nature of change in the countryside is (in the words of Blacksell and Gilg) "a relatively recent post-war development"; but, as Sheail argues, "the inter-war period was so critical and innovative that precedents can be found for almost every aspect of rural planning and conservation today". Together these two books offer a useful blend of historical perspective and identification of current problems, and they remind us that there is a rich vein of source material being tapped and yet to be tapped in studies of planning and the countryside in Britain. All of the authors are geographers: their approach, however, is widely based and of value to those concerned with economics, law, administration and other aspects of a complex subject. John Sheail, who is a historical geographer, has written his book as one of the Oxford Research Studies in Geography; Mark Blacksell and Andrew Gilg, both from Exeter University, have produced their volume as one of the Resource Management Series edited by Richard Munton and Judith Rees.

As a lawyer, I found that John Sheail's book offers a revealing analysis of the complicated and hesitant movement which culminated, after the Second World War, in comprehensive planning legislation dating from 1947. Against a background of the "economic and social malaise of the inter-war years" he examines a variety of experiments in local, regional and national planning secured through the initiative of legislators, officials and private pressure groups. In the country as a whole eloquent voices raised the spectre, in the words of Geoffrey Boumphrey (in one of the contributions to Britain and the Beast in 1938, a book which is mentioned by Sheail in his concluding chapter), of

the march of an inglorious suburbia across our countryside; the wanton sterilization of much of our most productive agricultural and marketgarden land; the marring of vista upon vista, where country still remains, by the erection of unsuitable buildings, by thoughtless felling of trees, by Philistine methods of road-making and road-widening — in short the blighting touch of the townsman upon the country.

The countryside was seen as threatened as never before by the "suburbanizing trend" reflected in the spread of the towns and of townspeople, in greater mobility achieved through modern traffic and new roads, in the search for new opportunities to enjoy the countryside for recreation or to adapt the countryside (allowing, for instance, for afforestation or new industrial growth) in the public interest.

One looks in vain for a consistent or positive approach in the inter-war years to the problem of reconciling the need for change and the desire to preserve. Rural conservationists succeeded in numerous battles but seemed a long way in 1939 from winning the war against what they saw as the evil of uncontrolled infringement of the old countryside. Sheail takes a balanced and understanding view of what they sought to achieve and how far they succeeded; and it is helpful to be told in the course of his book of the circumstances in which the Town and Country Planning Act 1932 was passed; of private legislation such as the Malvern Hills Act 1924 (later to be judicially considered, incidentally, by the House of Lords in Pyx Granite Co. Ltd. v Ministry of Housing and Local Government 11960| Appeal Cases 260); of the emergence of planning consultants and of the role of geographers in land-use surveys; of the work of such bodies as the Council for the Preservation of Rural England (founded in 1926), the Forestry Commission (established by statute in 1919) and the Youth Hostels Association (founded in 1930); of planning delays in the Ministry of Health and of "a somewhat ambivalent attitude" adopted towards statutory planning by the Ministry of Agriculture; and of numerous individuals (including Evelyn Sharp) who contributed in all sorts of ways to the national and local debates and controversies of the inter-war period. Drawing upon a wide range of original material, the author - admittedly on a selective basis, for the subject is vast manages to develop a carefully structured account of the problems of planning and conservation.

The same problems are with us today, though the emphasis may have altered and the statutory framework has changed out of all recognition. In their work, Blacksell and Gilg set out to assess "the impact of the policies and plans that have proliferated during the past thirty years to guide the process of change in the rural landscape of Great Britain". They detect many inadequacies in the present system, notably the absence of co-ordinated guidance in the process of change. We are told about the work of the Ministry of Agriculture, Fisheries and Food, the Forestry Commission, the Department of the the Countryside Environment. Commission and the Nature Conservancy Council; and a number of complex issues are explored, often on a basis of original material (particularly drawn from the authors' own case studies in Devon), on such topics as the nature and measurement of land use change, planning and land use, settlement planning, settlement change, and management in the countryside. For many readers a good deal of the authors' material will be unfamiliar, but there is clearly sufficient evidence of the value of the attempt by the Resource Management Series editors to provide "an interdisciplinary vehicle for major contributions from scholars and practitioners with a wide variety of academic backgrounds".

Problems of planning and conservation obviously loom large in a small and overcrowded country such as Britain - where the population, as Blacksell and Gilg point out, rose from 38.2 million in 1901 to an estimated 56 million in 1976. A study of the countryside alone becomes even more complex when one considers issues of pollution and the new and urgent demands for energy. Within their own terms of reference, these two books are important contributions to a subject of immense difficulty, riddled with conflicting interests, and they do much to correct what has perhaps been an overemphasis on town as opposed to country planning in the system which has evolved painfully over so many vears.

D.G.T. Williams is President of Wolfson College, Cambridge, and was formerly a member of the Commission on Energy and the Environment (1978–1981) and the Clean Air Council (1971–1979).

# **Enshrouded by prose**

**David Davies** 

Cosmos and Creator. By Stanley L. Jaki. Pp.168. Pbk ISBN 0-7073-0285-4. (Scottish Academic Press: 1981.) £3.25.

PROFESSOR Jaki, a Benedictine priest with a long-time interest in the theological implications of science, has written Cosmos and Creator to make available the major points in his other books on creation "in a concise form for the wider public". In this I believe he flatters the wider public (in which I include myself) with a breadth of learning that it does not possess. A sentence such as

the presence of nominalism is merely nominal in the thought of Buridan and Oresme, while the presence there of a pre-Ockhamist traditional notion of creation is rather robust

is a typical example of some of the heavy weather encountered; I, for one, was grateful to my Fontana Dictionary of Modern Thought for providing the glossary that Jaki should have included. And even when all the words are easily understood, the author writes too often in an obscure way for my taste.

No less revealing should it seem that in Eddington's and Jeans' success of making a fad (very superficially, to be sure) [of] the inference to God from science, the most effective part was played by a factor, apparently not at all mathematical. . . .

is a bit of a mouthful, particularly as the least of the many sins for which Jaki castigates Carl Sagan is for being a "consummate artist of sentences resting on double, triple and at times quadruple negatives".

Professor Jaki carries his argument through five chapters which read like five separate essays. In the first, "An Uneasy Fashion", he traces twentieth-century fashions in cosmology and the variable willingness amongst cosmologists to look through their specialization to the question of creation and a creator. His own view is that "he who says cosmos must say Creator in the traditional sense if man's sense of reality, purpose and consistency deserve more than lip-service", and he gives short shrift to some of those who have thought differently. Of course, proponents of the steady-state theory came into this category continuous creation was "the most glaring trick ever given scientific veneer".

The next chapter begins with a text, or rather an anti-text from Anatole France — "The universe which science reveals to us is a dispiriting monotony. All the suns are drops of fire and all the planets drops of mud". Jaki rather ponderously rises to this bait by emphasizing the beauty of science, and then goes on to discuss whether the universe is necessary or merely contingent: the climate of thought in our time is not at all favourable for a recognition of reason's ability to bring within sight the contingency of the universe and its raison d'être, its having been created by a Being truly necessary.

Jaki then turns to look at creation from the viewpoint of Christian theology — not just as a vague general belief but as a dogma or proposition which demands unconditional assent. The Fontana Dictionary says that "dogma" is today "mostly used pejoratively, to mean an opinion held on grounds, and propagated by methods, which are unreasonable". I think that most scientists who wade their way through this difficult chapter, with its assertion that the first chapter of Genesis is the classic statement of the dogma of creation, will incline to the same view, and retreat to the position that religion is mainly an ethical matter and on many dogmatic matters they can at best be agnostic.

Following a chapter on the philosophical status of books, the relevance of which to the general thread of the volume escapes me even after four readings, Jaki concludes with a discussion on extraterrestrial intelligence — "this ultimate extension of Darwinism and an utterly self-defeating exercise in wishful thinking". Darwinism itself is for him a belief in the meaningless of existence and much to be reviled, although he does believe in evolution as an "imperfectly understood instrumentality of a species in the rise of another". Present-day advocates of taking ETI seriously look in it for a "final rebuttal of supernatural

revelation", and come in for some predictable stick. On the other hand, theists, for whom "intellects are a special creation of God", not a mere epiphenomenon of biochemical diversification, can keep an open mind about ETI and even look forward to a possible encounter with other intellects because both sides "will know something of a universal brotherhood based on a common dependence on the Creator".

I have read this book from a scientist's perspective, and maybe it looks different from a theologian's. But, much as it has

made me think, got under my skin and in some places stimulated me, I feel ultimately that it has helped me disappointingly little. The opacity, the dogmatism, the verbal tricks all speak to me of a failure by the author to reach out and understand his "wider public" and hold a helpful dialogue. Professor Jaki, it seems to me, wants to tell rather than to help. I think most of us need help.

David Davies is Director of the Dartington North Devon Trust. He was Editor of Nature from 1973 to 1979.

# A population problem for archaeologists

Colin Renfrew

Demographic Archaeology. By F.A. Hassan. Pp.298. ISBN 0-12-331350-3. (Academic: 1981.) \$32, £21.20.

FIFTEEN years ago, following the publication of Ester Boserup's stimulating work The Conditions of Agricultural Growth (Aldine, 1965), geographers and archaeologists fully realized that population, or population density, must no longer be regarded as a highly dependent variable, fixed or at least rigidly limited in Malthusian manner by environmental constraints. On the contrary, the mode of exploitation of resources could be varied according to need, and the level of population came to be seen rather as an independent variable (although not an unconstrained one) which would in part govern the way in which a society would intensify its agricultural production.

Since that time, demographic arguments, some of considerable sophistication, have loomed large in archaeological explanations. Their proponents have been encouraged by the new rigour of archaeological survey procedures, which have moved on from the casual serendipity of the weekend outing or the summer safari to the often exhausting demands of probabilistic sampling strategies and intensive field-walking by disciplined survey teams.

Demographic Archaeology undertakes a comprehensive review of demographic thinking and population models in contemporary archaeology. It ranges from the consideration of the population density of hunter-gatherer groups, where the context is very much one of biogeography, through the impact of sedentism and food production upon carrying capacity, and on to the emergence of complex, urban civilizations with their large population centres. As a general survey it succeeds admirably in bringing out the central role of demographic argument in much contemporary archaeological thought, and in summarizing the rather formidable range of quantitative formulations which have already been put forward.

One central weakness in the whole subject area, however, is that early population figures, for any given time and place, are extremely difficult to arrive at, based as they almost invariably are on fragmentary survey data, supplemented by the incomplete excavation of a few settlement sites. At present this often restricts an estimate of the population variable from rising beyond the purely notional.

The crucial question of estimating population size from archaeological data takes up only one 32-page chapter of Hassan's book. And while this chapter is a very competent review of what has been written, it is not a very critical one, nor does it, to my mind, bring out the still unresolved difficulties in estimating population figures with any degree of accuracy and reliability from archaeological data. Until these difficulties are overcome, the demographic explanation in archaeology must remain something of a will o' the wisp: an enticing hypothesis, the testing of which remains a frustrating task. Hassan might have dealt at greater length with this crucial problem, for it is in the improved estimation of early population figures that the future of demographic archaeology must lie, rather than in the production of ingenious theories which, however attractive and plausible, have merely the status of speculation until sustained by acceptable data from the field.

Despite this central difficulty, which is not Hassan's problem alone but one which faces all researchers, his book is undoubtedly a sustained and coherent contribution to archaeological theory. It serves to bring together a whole series of ideas never before so effectively related, and takes its place at once among the small number of books on archaeological theory which rise above mere polemic to serve as valued works of reference.

Colin Renfrew is Disney Professor of Archaeology at the University of Cambridge.



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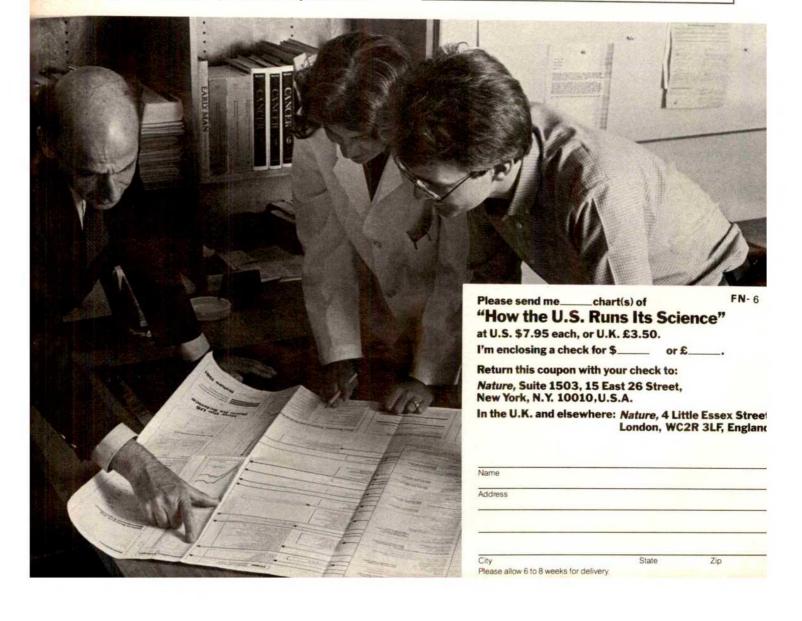
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Send résumé and three reference letters before Jan 15 to: Dr F Bellino, Dept Biol Sci, 336 Cooke, SUNY/Buffalo, Buffalo NY 14260. An Equal Opportunity/Affirmative (NW173)A Action Employer.

# IMMUTRON INC.

# ALLERGY RESEARCH

Positions are now open for Immunochemists (PhD) in the following areas: allergen specific IgE, basophil histamine release, allergen qulaity control and standaridization.

Candidates should have a strong background in at least one of these

Send CV to Irene B Haydik MD, Immutron Inc., Monrovia Ave, Newport Beach, Ca 92663 (NW171)A

# RESEARCH **BIOCHEMIST, Ph.D.**

The Lilly Research Laboratories, a leader in pharmaceutical and agricultural research, seeks a biochemically oriented scientist to expand its fermentation screening program. To be considered for this position, applicants must have a Ph.D. in biochemistry, enzymology, or a related area. The successful applicant will conceive, develop, and implement novel screening methods for the detection of nonantimicrobial activities in fermentation broths. These methods may include enzyme inhibition screens, tissue culture, or other techniques short of whole animal studies. The position requires an ability to work in a multidisciplinary environment that encompasses chemical, biochemical, and biological skills. A working knowledge of mammalian physiology would be a definite asset.

Eli Lilly and Company offers an excellent compensation program and apportunities for professional growth. Qualified individuals should send their résumé in confidence to

> Mr. Mark J. Tumey Personnel Representative Lilly Research Laboratories 307 East McCarty Street Indianapolis, Indiana 46285



Lilly Research Laboratories A Division of Eli Lilly and Company

An Equal Opportunity Employer, M / F

(NW155)A

# THE ROWETT RESEARCH INSTITUTE Bucksburn, Aberdeen AB2 9SB

# Appointment of DIRECTOR

Applications are invited for the post of Director of the Institute on the retirement of the present Director, Sir Kenneth Blaxter, FRS. The appointment can be taken up towards the end of 1982.

The main purpose of the Institute is to conduct research in animal nutrition and allied sciences, including the study of metabolic processes within animals, with particular emphasis on farm livestock. Some of this research also has relevance to human nutrition, and there may be a limited increase in this work.

The research programme is commissioned by the Department of Agriculture and Fisheries for Scotland, with advice from the Agricultural Research Council. The Institute forms part of the Agricultural Research Service and has close links with the University of Aberdeen, particularly its Schools of Medicine and Agriculture.

The Director is responsible to the Governing Body for the Administration of the Institute and management of the research programme which consists of eleven divisions. The director provides scientific leadership to the staff which numbers about 315, of whom 200 are in the scientific category. It is usual for the Director to be appointed Consultant Director of the Commonwealth Bureau of Nutrition, which is located at the Institute.

The post is graded Chief Scientific Officer (Lower Band) and there is a non-contributory superannuation scheme. During the tenure of the appointment a detached house sited within the Institute campus may be rented by the successful candidate.

QUALIFICATIONS: Applicants should have appropriate qualifications, a distinguished record of personal research and experience of management of research.

Further particulars are available from the Secretary of the Institute, to whom applications should be submitted with full curriculum vitae and the names of three referees. Closing date; 5th February 1982.

# PUBLIC HEALTH LABORATORY SERVICE BOARD

CENTRAL PUBLIC
HEALTH LABORATORY
VIRUS REFERENCE LABORATORY
BIOCHEMIST

A postdoctoral biochemist is required in the Virus Reference Laboratory to join a group of microbiologists working on viral gastroenteritis. The work will involve the biochemical characterisation of viruses associated with nonbacterial gastroenteritis and a study of the possible relationship of the human agents with viruses of veterinary importance. Experience in the purification of viruses would be an advantage.

The successful candidate will be appointed as a Senior Grade Biochemist according to qualifications and experience; the salary scale for which is £8,691—£11,073 inclusive of London Weighting.

NHS Terms and Conditions of Service apply.

Applycations to Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. Telephone number 01-205 7041.

Closing date: 8th January 1982. (076)A

# UNIVERSITY COLLEGE OF NORTH WALES, BANGOR RESEARCH ASSISTANT BACTERIAL PLASMID ANALYSIS

Applications are invited for the above post, to collaborate with Dr N J Grinter (ext 359) on studying mechanisms governing the accurate partitioning of bacterial plasmid molecules at cell division, including the isolation and mapping of plasmid mutations and DNA base sequence analysis.

Applicants for this post, which will be for one year in the first instance (renewable for a further two years) and within the range: £5,285—£7,700 per annum, should be at least biological-science graduates, with a knowledge of bacterial genetics and the biology of bacterial plasmids. Experience of recombinant DNA technology would be an advantage.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of two referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, by not later than 11th January, 1982. (057)A

# COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES Colorado State University

DEPARTMENT OF MICROBIOLOGY

### HEAD

Applications and nominations are invited for the position of Head of a progressive and diversified Department. Qualifications required are an earned doctoral degree (PhD, DVM, MD or equivalent), distinguished achievement in research and teaching and demonstrated capacity for leadership. Research interests of the Department include virology, immunology, bacteriology and mycology at the molecular, veterinary, medical and environmental levels. The Department is responsible for undergraduate, graduate and veterinary programs in microbiology. Deadline for receipt of applications is February 15, 1982.

Candidates should submit a curriculum vitae to; D Robertshaw, Chairman, Search Committee, Department of Physiology and Biophysics, Colorado State University, Ft. Colins, Colorado 80523. Colorado State University is an EEO/AA employer. E O Office, 315 Student Services Building.

(NW167)A

PHYSIOLOGY Faculty Position: Assistant Professor tenure-tract position for July, 1982. An interest and post-doctoral research experience in gastrointestinal physiology, particularly in peptides of the gastrointestinal tract and their regulatory role, is preferred. Candidates will be expected to teach physiology of the digestive tract to medical students and to participate in graduate courses and to develop an independent research program. Applicants should submit curriculum vitae, reprints and statement of research interests, and teaching experience, and have three letters of recommendation sent to: Dr Robert E Forster, Chairman, Department of Physiology, School of Medicine, University of Pennyslvania, Philadelphia, Pa 19104. An equal opportunity/affirmative action employer. (NW164)A

# THE INSTITUTE OF DERMATOLOGY (University of London) ELECTRON MICROSCOPY UNIT

requires a pre- or post-doctoral Research Assistant to join a small team investigating mechanisms in blistering diseases. Methods in use include transmission EM, immunohistochemistry and cell culture.

The post will be for 2 years in the first instance. Salary according to age and experience (1A scale for applicants with PhD).

Applications, including CV and names of two referees to: Dr R A J Eady, Institute of Dermatology, Homerton Grove, London E9 6BX. (Tel: 01-985 7061 ext 66) from whom further details may be obtained. (074)A

# UNIVERSITY OF LEICESTER DEPARTMENT OF GENETICS POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from persons with a good background in biochemistry or molecular genetics for a postdoctoral research position financed by a Cancer Research Campaign award. The project will be concerned with the characterisation of error-prone DNA replication in E. coli induced by carcinogens or UV-irradiation. This will involve the use of cloning teachniques, in vitro DNA replication systems and enzyme purification procedures. The work is likely to involve close collaboration with research groups in the Department studying other aspects of the SOS response in E. coli and with groups studying the control of and mechanism of plasmid DNA systhesis using an in vitro system.

The appointment will be tenable for up to 3 years from 1 January 1982 or as soon after as can be arranged. Starting salary £6,880 (at age 26) per annum with full superannuation provision.

Applications with names of two referees to Dr I B Holland, Department of Genetics, University of Leicester, Leicester LE1 7RH.

(043)A

# **ORGANIC CHEMIST**

A post doctoral research fellow is required for one year to join a team studying the chemistry of tobacco. The work offers interesting structural and synthetic problems in the natural product field, which requires knowledge of both modern spectroscopic and chemical methods and advanced separation procedures.

The annual salary is £11,000 and assistance will be given with relocation expenses and in obtaining accommodation.

Please, write to Professor C R Enzell, Research Department, Swedish Tobacco Company, PO Box 17007, S-104 62, Stockholm, Sweden, (Telephone 08-69 09 00) enclosing a brief curriculum vitae and references, before January 20, 1982. (W537)A

# UNIVERSITY OF SASKATCHEWAN

DEPARTMENT OF BIOLOGY

Postdoctoral position available to investigate the liposome-mediated transfer of chromosomes into auxotrophic cells of *Datura*.

Candidates should have a strong background in genetics and cell biology and be trained in the use of plant cell culture techniques.

The position is available immediately for a one-year term (renewable for a further two years) at a salary of \$16,380 Can.

Candidates should send curriculum vitae and two letters of recommendation to Dr John King, Department of Biology, University of Saskatchewan, Saskatoon, Sask, Canada S7N OW0. (NW153)A

# THE UNIVERSITY OF MANCHESTER RESEARCH ASSOCIATE IN **EXPERIMENTAL NUCLEAR** STRUCTURE PHYSICS

Applications are invited for this post, Applications are invited for this post, tenable from February 1, 1982 for up to three years. The appointee will pursue research at the Nuclear Structure Facility, a 30 MV tandem Van de Graaff which is nearing completion at the Daresbury Laboratory. Duties also involve assistance with the commissioning of the isotone separator at the the isotope separator at the Daresbury Laboratory.

Applicants should hold a PhD and have an aptitude for research in experimental physics. Initial salary pa; £7,290.

Applications, with full CV and names of two referees to: Dr R Chapman, Department of Physics, the University, Manchester M13 9PL from whom further details may be obtained. (075)A

# NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING MICROBIOLOGY DEPARTMENT A SCIENTIFIC OFFICER

is required for a period of 2 years to assess and develop rapid sensitive methods for detecting any traces of antibiotic in milk. A wide range of microbiological, immunological and radiometric techniques will be involved.

Applicants should have a first or second class (2:1) honours degree in Microbiology, Biochemistry or Immunology.

Appointment, for a limited period of only two years, will be as Scientific Officer; salary scale £5,176 to £6,964. Non-contributory superannuation.

Apply in writing, enclosing full cv, to the Secretary, NIRD, Shinfield, Reading RG2 9AT.

Quote reference 81/30. (061)A

# **MOLECULAR CELL BIOLOGY PREDOCTORAL FELLOWSHIPS**

The Graduate Program in Molecular Cell Biology invites applications from highly qualified students interested in an interdisciplinary PhD program. Major areas of emphasis include animal virology, bacterial and somatic cell genetics, cancer biology, cellular immunology and immunochemistry, connective tissue and protein biochemistry, infectious diseases and host defense, membrane biochemistry, nucleic acid synthesis, and research involving the cause and prevention of dental caries.

All students admitted to the program receive stipends of \$6,300 plus tuition and fees per year. After admission to candidacy the stipend is increased to \$7,000 per year.

Contact: Dr Roy Curtiss III, Director of Molecular Cell Biology Graduate Program, Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294. (NW165)A

# THE UNIVERSITY **OF TASMANIA CHAIR OF CHEMISTRY**

Applications are invited for appointment to the Chair of Chemistry, which will become vacant on the retirement of Professor H Bloom on 31 December 1981.

Applications are invited from candidates in any field of Chemistry.

The present professorial salary is \$A41,509 pa.

Candidates who wish to apply should in the first instance write to The should in the first instance write to The Registrar, University of Tasmania, Box 252C, GPO, Hobart, Tasmania, Australia 7001, or to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, to obtain a detailed statement of the conditions of appointment and conditions of appointment and application forms.

Applications close on 19 February (078)A

# UNIVERSITY OF ESSEX DEPARTMENT OF BIOLOGY RESEARCH OFFICER

Applications are invited for the post Research Officer funded by the on Research Officer funded by the Science and Engineering Research Council (salary on Range IB £5,285 — £7,700). The appointment to commence in January 1982. The research project is planned to develop a representation planned to develop a transformation system in Aspergillus nidulans. This system will be used for cloning, recombination studies and identification of transposable sequences. The successful applicant will work under the direction of Dr C Scazzocchio and will be eligible to read for a higher degree. Candidates requiring further information should contact Dr Scazzocchio. Telephone: Colchester (0206) 862286 ext 2427.

Applications in writing as soon as possible quoting reference AG/131/ N/S to Dr C Scazzocchio, University of Essex, Wivenhoe Park, Colchester CO43SO.

# DIRECTOR

The Herbert Whitley Trust and the Paignton Zoological and Botanical Gardens Ltd will shortly require a Director owing to the forthcoming retirement after some 25 years' service of the Managing Trustee and the General Manager.

The Trust, a registered Charity, owns the Zoo, the Slapton Ley Nature Reserve and other properties.

Candidates should have zoo experience, scientific training, commercial flair, an interest in education and the ability and determination to take forward two well respected organisations to even greater prestige and value to the community in the next 20 or 30 years.

Apply in writing to Managing Trustee, Herbert Whitley Trust, Primley Estate Office, 190 Totnes Road, Paignton, Devon TQ3 3SB (tel. Paignton (0803) 558189).

(071)A

# CELLTECH

# ASSAY SYSTEMS RESEARCH

Celltech is a new British Company backed by Government and City financial institutions, and is dedicated to the exploitation of discoveries in the field of biotechnology. Celltech is currently situated in Slough, but will transfer to a new permanent location at a later date.

Two appointments are to be made in a newly formed research group investigating novel immunoassay systems.

Ref 121 PHD: Senior Scientist grade required, with 1-3 years post doctoral experience in the field of immumoassay. The successful candidate would be expected to show an innovative approach to the development of new assay systems which exploit the advantages of monoclonal antibodies.

Ref 122: Senior Technician with HND, M I Biology of similar qualifications and several years experience in immunological R and D, required to participate in the development of novel immunoassay systems.

Celltech offers excellent salaries, subsidised lunches, contributory pension scheme and 4 weeks annual holiday.

Applicants should send a cv to the Personnel Department, Celltech Limited, 244 Bath Road, Slough, Berkshire. (080)A



# THE CALIFORNIA INSTITUTE OF **TECHNOLOGY**

invites applications for

# JUNIOR FACULTY POSITIONS IN ASTRONOMY

Caltech wishes to appoint two tenure track assistant professors, although in exceptional circumstances successful candidates may be offered associate professorships without tenure. The major research interests of candidates should be in observational optical or infrared astronomy. They will be expected to devote the major fraction of their effort to observational work in instrument development at Palomar Observatory which is operated by Caltech.

Applications should include (1) a description of educational background; (2) a résumé of research and teaching experience; (3) a list of all publications; (4) copies of all manuscripts submitted for publication but not yet published; (5) a statement of current research interests and future research plans. The applicant should ask three scientists who know him/her personally to send letters of evaluation directly to Professor M H Cohen, Executive Officer for Astronomy, California Institute of Technology, Pasadena, CA 91125. Completed applications and letters of evaluation should be received by March 1, 1982; appointments will be made as soon thereafter as circumstances permit.

Caltech is an Equal Opportunity Employer with an Affirmative Action Program. We encourage applications from qualified women and members of minority groups. (NW166)A

# COMMONWEALTH AGRICULTURAL BUREAUX Three vacancies for **ENTOMOLOGISTS** in the COMMONWEALTH INSTITUTE OF **ENTOMOLOGY** (IDENTIFICATION

SERVICE)

Duties: To carry out identifications, and investigate the taxonomy, of insects in either the Hemiptera, Hymenoptera or Diptera. Appointees will be members of the Institute's Identification Service located in the British Museum (Natural History) in South Kensington, and will work under the supervision of senior taxonomists as appropriate. Posts probationary for the first year.

Qualifications: A degree or equivalent qualification in a relevant subject with emphasis on entomology, and evidence of an interest in, and aptitude for, taxonomic work.

Selary: In the Taxonomist (£5,176 - £6,964) or Higher Taxonomist (£6,530 - £8,589) scale, plus Inner London Weighting of £1,087 per annum; possibility of eventual progression to higher scales. Starting salary according to age, qualifications and experience.

Application forms and further particulars from the Director, Commonwealth Institute of Entomology 56 Queen's Gate, London SW7 5JR, England.

Closing date for receipt of applications: 31st March 1982.

(063)A

# SHARE OUR FUTURE

# **RESEARCH MANAGER**

Edmonton — This is a senior management position with responsibility for developing a research support capability for the Department. You will work in an active team environment to develop and manage research programs and projects relevant to Departmental policy. You will review and evaluate research proposals, and reports, communicate research information to Departmental staff and represent the Department on University, government and industry committees. You may also undertake research in your area of specialization. Qualifications: PhD in plant physiology ecology and considerable experience in environmental research and management plus a good general knowledge of environmental sciences and problems, specifically effects of pollutants on ecosystems. Equivalencies will be considered.

Salary: Up to \$42,696. Closing Date: Dec. 30, 1981. Competition // EV M642-4. Environment

Please send an application form or resume quoting competition number to:

Alberta Government Employment Office 5th Floor, Melton Building 10310 Jasper Avenue Edmonton, Alberta T5J 2W4

(NW174)



# THE UNIVERSITY OF LEEDS DEPARTMENT OF RADIOTHERAPY

Applications are invited for a post of POSTDOCTORAL

RESEARCH FELLOW in the Department of Radiotherapy, Cookridge Hospital for work on biology and immunology of tumour

cells involving the use of flow cytometric techniques.

A good honours degree is biochemistry/biology and a PhD in biochemistry or cell biology are

The post is available from 1 January 1982 for a fixed period of up to three

required.

Salary on the IA scale for Research and Analogous Staff (£6,070 — £10,575), according to age, qualifications and experience.

Informal enquiries may be made to Dr J E D Dyson at Cookridge Hospital, Leeds (telephone Leeds 673411 ext 219).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 108/1/D. Closing date for applications 7 January 1982. (081)A

# SCLAVO/ITALY POSITIONS IN MOLECULAR BIOLOGY

Sclavo, an ENI subsidiary, is a company specialized in the production of biological products for medical application.

Headquarters, plants and applied research facilities are located in Siena in a very pleasant situation, and laboratories are modern and well equipped. The company is now organizing a separate center for basic research. This center includes divisions of molecular biology, biochemistry, cell biology and immunology.

Positions are available in the department of Molecular Biology which is interested in gene expression in mammalian cell systems. Applicants are expected to have a productive research record in the fields of nucleic acids, genetic engineering, cell transformation or virology, to join ongoing research programmes on mammalian cell differentiation. Salaries will be commensurate with experience and seniority of the applicants, in accordance with international standards.

Send résumé to: Prof. Paolo Neri, ISVT Sclavo SpA, Via Fiorentina, 1, 53100 Siena, Italy. (W538)A

# GLOUCESTERSHIRE AREA HEALTH AUTHORITY

# MEDICAL LABORATORY SCIENTIFIC OFFICERS/JUNIOR

Medical Laboratory 'B'
Gloucestershire Chemical Pathology Service

The Chemical Pathology Department is sited in and serves the two district general hospitals in Cheltenham and Gloucester covering a population of 500 000

The laboratories are well-equipped and offer a comprehensive service. Further study is encouraged.

Applicants for the Medical Laboratory Scientific Officer grade must hold an HNC/HND in MLS (clinical chemistry) or appropriate science degree and be registered with the Council for Professions Supplementary to Medicine.

The Junior 'B' grade is open to holders of an ONC/TEC in Sciences (medical laboratory science variant) or science degree. Informal visits are welcomed by arrangement.

Application forms and further information may be obtained from Mr I.R. Holliday, Principal Medical Laboratory Scientific Officer, Department of Clinical Pathology, Gloucestershire Royal Hospital, Great Western Road, Gloucester GL1 3NN. (067)A

# COLLEGE OF ENGINEERING AND APPLIED SCIENCE

The University of Wisconsin-Milwaukee invites applications and nominations for the position of Dean of the College of Engineering and Applied Science. The University, with 27,000 students, is one of two doctoral level campuses in the University of Wisconsin System with a strong emphasis on graduate education and research. UWM is located in the heart of the state's largest metropolitan area and the center of its manufacturing and business enterprise.

The College of Engineering and Applied Science has five departments: Civil Engineering, Electrical Engineering and Computer Science, Industrial and Systems Engineering, Materials Engineering, and Mechanical Engineering, offering programs leading to bachelor through doctoral degrees.

The Dean is responsible for administrative leadership of the College. Duties include the continuing development of ongoing research programs, liaison with the industrial community, and promotion of continuing professional education programs.

Candidates should have a doctoral degree in engineering or in an applied science or demonstrated equivalent, and qualifications for a tenured faculty appointment in one of the departments of the College. The position will be available by the Summer of 1982.

All nominations and applications for the position (including résumé and the names of at least three references) should be postmarked by March 1, 1982, to: Dr Robert C Eidt, Chairperson, Search and Screen Committee, The University of Wisconsin-Milwaukee, Department of Geography, Milwaukee WI 53201.

An Equal Opportunity/Affirmative Action Employer. (NW159)A

# UNIVERSITY OF ST ANDREWS

DEPARTMENT OF PHYSIOLOGY & PHARMACOLOGY

# POSTDOCTORAL NEUROBIOLOGIST

Applications are invited for a post of Postdoctoral Research Assistant to work on dendritic sprouting in invertebrate neurones. The work is financed by the SERC for three years from March 1982 or as soon as possible thereafter. Salary within the range £6,070 to £6,880 per annum, according to age and experience, plus superannuation.

For further information about the work please contact Dr R M Pitman, Department of Physiology and Pharmacology (telephone St Andrews 76161 ext 7209).

Applications with curriculum vitae (two copies preferably in typescript), and the names of three referees should be submitted to the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ by 15th January, 1982.

. (077)A



# I.L.R.A.D. (Nairobi)

Applications are invited from suitably qualified candidates for the post of:-

# POST DOCTORAL FELLOW Ref No PDF/L4/81/1

A position is available for a post-doctoral fellow to work with the Immunobiology Unit on the definition of cells of the bovine immune system. Applicants must have experience in preparing, maintaining and testing, functional T-cell, natural killer cell etc., clones from a mammal.

The successful applicant will be expected to work closely with other members of the unit who have produced monoclonal antibodies against bovine B-cell T-cell and macrophage surface membrane determinants. The studies are directed towards understanding bovine immune response against African trypanosomes and *Theileria* infected bovine cells. This is an international position, the salary will be paid in U.S.\$ and the successful applicant will be assisted with moving expenses.

Applications identified with Ref No including C.V., summary of PhD thesis, and three references should be sent to:-

Chief Personnel Officer, I.L.R.A.D P.O. Box 30709, NAIROBI, Kenya

Closing date: 7/1/82

(W528)A

# UMIST

DEPARTMENT OF BIOCHEMISTRY
Ref: HIO/149/AI
POST-DOCTORAL
PHARMACOLOGIST/
NEUROPHYSIOLOGIST

required to work on a two year industrially funded project investigating nocrotransmitters and receptors with a view to developing new insecticides. Experience in intracellular recording would be an advantage. Informal enquiries to Dr N M Tyrer (061 236 3311 ext 2115 or 2111)

Salary will be on the scale £6,070 — £7,700 per annum.

Requests for application forms, quoting the above reference should be sent to the Registrar, Room B6, UMIST, PO Box 86, Manchester M60 1QD. The closing date is 31 December, 1981. (082)A

# ROSWELL PARK MEMORIAL INSTITUTE MOLECULAR IMMUNOLOGY

a comprehensive cancer research center has available three-tenure track positions which are open in the Department of Molecular Immunology. The department is expanding into the areas of idiotype manipulation and recombinant DNA technology. One of the positions is at the senior, (full professor equivalent) level and two are at the junior level. No formal teaching is required at the Institute, but graduate students are available through the institutional graduate program. The candidate for the senior staff position will be consulted in the selection of the junior candidates.

Applications should be forwarded to Heinz Kohler, Chairman of the Department of Molecular Immunology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14263. An Affirmative Action/Equal Opportunity Employer. (NW170)A

# POSTDOCTORAL RESEARCH ASSOCIATE

Position available immediately for a period of two years for collaborative work in the field of cell motility. Interests of the laboratory are in immunocytochemical analysis of contractile proteins in the cytoplasm and nucleus and microinjection of fluorescent proteins into cells. Individuals with experience in antibody and/or protein purification preferred. Salary range is \$15-17,000/year.

Please send résumé and 3 letters of reference to Dr J W Sanger, Department of Anatomy/G3, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104. USA.

The University of Pennsylvania is an Equal Opportunity Employer. (NW158)A

# UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF

PHARMACOLOGICAL SCIENCE AND CLINICAL BIOCHEMISTRY RESEARCH ASSOCIATE

Applications are invited from candidates with an interest in metabolic biochemistry for a post-doctoral post of Research Associate to participate in a project on the toxicology of compounds which influence intermediary metabolism directed by Dr H S A Sherratt and Professor K G M M Alberti, which is supported by the Wellcome Trust. The post is available for two years from 1st February 1982. Starting salary £6,070 per annum on the Range 1A scale.

Applications, naming two referees, should be submitted as soon as possible to Dr H S A Sherratt, Department of Pharmacological Sciences, The Medical School, University of Newcastle upon Tyne, Queen Victoria Road, Newcastle upon Tyne NE1 7RU, from whom further information can be obtained, tel: 0632 328511 ext 3031. (058)A

# THE TECHNICAL UNIVERSITY OF DENMARK ASSISTANT/ASSOCIATE PROFESSOR "APPLIED MICROBIAL GENETICS"

At the Department of Microbiology a position as assistant/associate professor is to be filled from the 1st of May, 1982 or later. The successful candidate will assist in the establishing of a new group in "Applied Microbial Genetics". The group will be responsible for teaching and research within this field (recombinant DNA technology, host-vector systems, optimalization of gene expression, genetics of industrial microorganisms, risk assessment, etc.). Applicants with experience in molecular biology, genetics and biochemistry, preferably with industrial microorganisms, will be given preference.

The applicant should be able to develop independent research projects and is expected to cooperate with existing research teams. Furthermore, the applicant should have an appreciation of the social implications of this field in accordance with the report having formed the basis of the establishment of this field. This report may be obtained from the Secretariat of the Faculty (telephone 452-882222 ext 2237).

Further information may be obtained from Professor Kaspar von Meyenburg, Department of Microbiology, Building 221, Technical University of Denmark, 2800 Lyngby (telephone 452-884066 ext

The salary is fixed according to the agreement between the Ministry of Finance and the relevant trade union. Details on the mode of employment especially with regard to the first year may be obtained from the Secretariat of the Faculty.

The application should include curriculum vitae, relevant publications, if possible in 3 copies, and a detailed account of teaching experience.

The application is to be addressed to: The Faculty Council of Chemical Sciences, Technical University of Denmark, Building 101A, 2800 Lyngby, Denmark.

The application should be to hand for the University not later than the 5th of February, 1982. (W536)A

# THE INSTITUTE FOR ADVANCED STUDY

will have several openings for members in theoretical physics and astrophysics for the academic year 1982-83. The positions are at a post-doctoral or higher level and applicants will be selected on the basis of their ability to do research in the areas of elementary particles, mathematical physics, astro-physics, plasma physics, general relativity and statistical mechanics. Preference is given to candidates who have received their PhD within the last year or two.

Postdoctoral members frequently collaborate with each other, with faculty members at the Institute or Princeton University, and with researchers at other institutions.

Appointments are usually for no more than two years and support is typically full salary for postdoctorals and half salary for more senior persons. Women and minorities are encouraged to apply.

For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW1006)A

# UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF CLINICAL BIOCHEMISTRY AND METABOLIC MEDICINE

# RESEARCH ASSOCIATE (BIOCHEMIST)

Applications are invited for a temporary post-doctoral biochemist Research Associate in the above department. The successful candidate will work closely with the Head of Department in a research group supported by the British Diabetic Association investigating the effects of experimental diabetes and its treatment on intermediary metabolism. Experience in intermediary metabolism, enzymology, enzyme activation, pancreas transplantation or mechanisms of hormone action would be advantageous. The appointment is available from 1st February, 1982 and will be for three years. Salary on the Range IA scale £6,070 — £10,575 per annum.

Applications, giving full curriculum vitae and two referees, should be submitted within two weeks of the appearance of this advertisement to Professor K G M M Alberti, Department of Clinical Biochemistry and Metabolic Medicine, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, from whom further information can be obtained (tel: 0632 328511 extension 3132).

(059)A

# HILL FARMING RESEARCH ORGANISATION

# Scientific Officer

Applications are invited to fill a Scientific Officer vacancy at our Sourhope Research Station. The station is situated approximately seven miles from Yetholm in Roxburghshire.

The successful appointee will assume overall responsibility for and provide assistance with collection and submission of all experimental data to Headquarters. They will also have some responsibility for the collection and transmission of data from non-routine experimental work, act as deputy when the Officer in Charge is absent from the Station and assist or accept sole responsibility for looking after parties of visitors to the Station.

Qualifications: HNC or HND or a pass degree or equivalent in a scientific or mathematical subject. Preference will be given to candidates with a qualification in agriculture.

Salary: Depending on qualifications within the range £5,176 to £6,964 with £5,718 being the maximum starting salary.

Other conditions: Non-contributory superannuation scheme 20 days

annual leave. Under normal conditions 5 day week (42 hrs)

Application forms and further information may be obtained from the Secretary, Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY to whom they should be returned not later than 8th January 1981. Please quote ref A/6/265

# STRANGEWAYS RESEARCH **LABORATORY ENVIRONMENTAL CARCINOGENS GROUP** POST-DOCTORAL RESEARCH ASSISTANT

A Biologist with research experience in histo-pathology is required to work on carcinogenesis induced by industrial and environmental particulates. The programme is especially concerned with possible synergistic processes between mineral and metallic particles and fibres, and systemic carcinogens, all at low level.

The project is funded by the Health & Safety Executive, and the appointment will be for a 3 year period. It will be made in the MRC Non-clinical Scientific Grade II range with USS benefits at a Salary level depending on age and experience.

Curriculum vitae with 2 referees to: Administrative Secretary, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN.

# UNIVERSITY OF LONDON **British Postgraduate** Medical Federation

CARDIOTHORACIC INSTITUTE DEPARTMENT OF ALLERGY AND CLINICAL IMMUNOLOGY

# MEDICAL LABORATORY SCIENTIFIC OFFICER

Applications are invited for the above position available for at least two years. The successful candidate will be involved in a research programme on the role of leucocytes in inflammatory and allergic responses in both humans and laboratory animals. Experience in biochemical/ immunological/parasitological techniques is desirable, and applicants should hold a relevant HNC or equivalent.

Application forms are available Application forms are avalable from The Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP. (Tel: 01-352 8121 ext 4163). Closing Date 15th January

# **ICHTHYOLOGY JOB** DESCRIPTION

Cornell University solicits applications for a tenure-track position, preferably an Assistant Professor, in the Section of Ecology & Systematics to begin September 1982. We seek a Systematic Ichthyologist with broad interests in vertebrate biology and a strong research program. The successful candidate will participate in curation of the Ichthyology Collection. Teaching responsibilities include an upper-level course, and participain the graduate program in vertebrate biology.

Applications must include a curri-culum vitae, names of three references, and will be reviewed February 1, 1982. Send application to: Dr W N McFarland, Section of Ecology and Systematics, Cornell University, Ithaca, New York 14850. Cornell University is an affirmative action/equal opportunity employer. (NW157)A

# UNIVERSITY OF ARIZONA DEPARTMENT OF CHEMISTRY **ACADEMIC POSITION**

Invites applications for a possible tenure-track position at the Assistant Professor level in one of the following areas: (1) Synthetic Solid State Chemistry; (2) Surface Chemistry; and (3) Atmospheric or Low-Temperature Geochemistry.

Candidates should have demonstrated in their Ph D and/or postdoctoral work the ability to develop a vigorous and innovative research program in one or more of the above areas and have a commitment to instructional excellence.

A résumé brief description of research plans, and three letters of recommendation should be sent to Professor William S Glaunsinger, Chairman, Search Committee, Department of Chemistry, Arizona State University, Tempe, Arizona 85287. EO/AA employer. (NW146)A

# THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S **HOSPITAL**

(University of London) West Smithfield, London EC1A 7BE

# POST DOCTORAL RESEARCH FELLOW

required in Department of Haematology for Cancer Research Campaign grant entitled "Isolation and Characterisation of regulatory variants for the expression of murine lymphocyte cell surface antigens". Experience in cell membrane biochemistry necessary and immuno-logy/cell culture experience desirable. The post is tenable for three years from 1 January 1982, or soon after. Salary on Scale £6,070 — £10,575 plus

Apply in writing with CV and names of two referees to The Secretary of the College at the above address, quoting ref 925. Further information from Dr M A Horton Tel: 01-600 9000 extension 3272. (021)A

# Southampton THE WINIVERSITY

# **INSTITUTE OF CRYOGENICS**

Applications are invited for the following appointments in the Industrial Advisory Unit:

### 1. Technical and **Projects Manager** 2. Research Scientist or Engineer

Candidates should be graduates in Science or Engineering and have working research experience in industry in either cryogenics or refrigeration engineering.

The Unit is an integral part of the Institute of Cryogenics and some teaching ability is required.

Salaries in range to £12,860 depending on qualifications and experience.

Experience.
Further particulars from D. A. S.
Copland, The University, South-ampton SO9 5NH, quoting reference no 319/A/N. (070)A

# LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

# FIELD PROJECT IN MALAWI ASSISTANT TO DIRECTOR

Required to work on a contract of not less than 3 years' duration for the Director of a long-term Leprosy Evaluation Project in Northern Malawi. The primary responsibility is in relation to quality control of data but some field work may be involved. Experience in Epidemiology, Statistics, management of social survey data or another relevant area is required. Candidates must be mature and be fully fit to serve in the tropics in rural conditions.

A salary of not less than £6,000 is envisaged together with free passages for the successful candidate (and spouse, if married). Tropical kit and overseas allowances are paid.

Applications, consisting of full curriculum vitae and the names and addresses of two referees, should be submitted to Dr P E M Fine, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, from whom further articulars may be obtained. (066)A

# UNIVERSITY OF DUNDEE

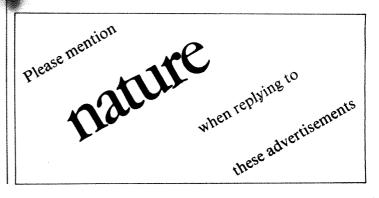
DEPARTMENT OF PHYSICS

### There are two vacancies for RESEARCH ASSISTANTS

to work for up to three years as members of a research group directed by Professor W E Spear, FRS and concerned with the development of amorphous silicon devices. The posts offer challenging opportunities to applicants with higher degree or professional qualifications in physics or electrical engineering and with some relevant research or development experience preferably in the amorphous semi-conductor field.

The starting salary for both posts will be in the range £5,285 — £6,880 per annum.

Applications containing full curri-culum vitae (3 copies) and the names of two referees should be sent by 7th January 1982 to the Personnel Office, The University, Dundee, DD1 4HN. Please quote reference EST/58/81J. (069)A



# AGRICULTURAL RESEARCH COUNCIL Poultry Research Centre, Roslin, Midlothian EH25 9PS **Poultry Scientist (Breeder Production)** HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

A Poultry Scientist (Breeder Production) is required to carry out applied research aimed at improving the productivity of breeding chickens and research aimed at improving the productivity of breeding chickens and turkeys. Initially, emphasis will be given to nutritional research but cooperation with other scientists in the Centre, to apply findings from different areas of basic science, such as Reproductive Physiology and Ethology, is expected. The appointee will be required to liaise with advisory and industry groups to ensure effective innovation in this programme and application of the findings.

Training in agricultural science, research experience and a demonstrable interest in applying scientific work to animal production are required. Computer modelling and other quantative methods are important in this

field and candidates should have relevant skills and/or interests.

Qualifications: First or upper second class honours degree in Agriculture, Agricultural Science, Applied Biology or related subject, with at least 2 years post-graduate experience; 4 years postgraduate experience is necessary for entry at Senior Scientific Officer level. A postgraduate qualification would be preferred.

Salary: In scale £6,530 — £8,589 at HSO level, £8,209 — £10,322 at SSO level. Non-contributory pension scheme. 22 days annual leave.

Applications forms are available from Mr. A. I. Menzies, Institute Secretary, at the above address. Completed forms must be (084)A returned to him by the 8th January 1982.

# UNIVERSITY OF BATH

DEPARTMENT OF PLANT BIOLOGY

# POSTDOCTORAL RESEARCH OFFICER

required for project on the freezepreservation of potato germplasm, supported by the Overseas Development Administration and directed by Professor G G Henshaw. Experience of tissue culture and/or cryobiological techniques would be an advantage.

Starting salary up to £6,880 according to qualifications and experience. Ref no 81/128.

# **EXPERIMENTAL OFFICER**

required immediately for above research project and associated work with potato tissue cultures. Applicants should have some experience of tissue culture or aseptic techniques and be qualified to at least HNC or equivalent.

Starting salary up to £5,762 according to qualifications and experience. Ref no 81/129.

Application forms and further details from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting the appropriate ref Closing date: 4.1.82. (072 (072)A

# ISTITUTO NAZIONALE DI FISICA NUCLEARE (INFN)

# **NUCLEAR AND PARTICLE PHYSICISTS**

Applications are invited for two research posts for one year, starting October 1982.

The first post is for a theorist working in intermediate energy nuclear physics, either at the Istituto di Fisica, Università di Genova, or at the Istituto di Fisica, Università di Cagliari.

The second is for a theorist working in elementary particle physics, with a specific interest in field theory and fundamental interactions.

Both positions are for non Italian citizens and are at a senior level.

Salary will be determined by the individual experience and present position of the applicant.

Send applications and requests for further information to: Prof Dr Antonino Zichichi, Presidente, National Institute of Nuclear Physics (INFN) Piazza dei Caprettari, 70 00186 Rome - Italy.

The application deadline is February 15, 1982. (W540)A

# **INVERTEBRATE PHYSIOLOGIST**

The Department of Biology of the Pennsylvania State University invites applications for a tenure-track position for an invertebrate physiologist to begin Fall 1982. A PhD degree is required. Preference will be given to applicants who interface strongly with ecological and evolutionary aspects of invertebrate physiology and who will interact with present faculty in the department. The successful candidate will be expected to develop a strong research program and teach an introductory undergraduate course and an upper division or graduate course in his/her area of expertise. Rank depends on qualifications and experience. Submit curriculum vitae, statement of research and teaching interests and three letters of recommendation by 15 February Invertebrate Physiologist, Search Committee, Department of Biology, 208 Mueller Laboratory, Box 4, Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802.

An Equal Opportunity/Affirmative Action Employer.

(NW162)A

# CHAIR **DEPARTMENT OF** ANATOMY & CELL BIOLOGY DOWNSTATE MEDICAL CENTER

Candidates and those wishing to nominate candidates are asked send a curriculum vitae to Dr Bernard Jaffe, Chairman, Search Committee, Downstate Medical Center, Box 40, 450 Clarkson Avenue, Brooklyn, NY 11203.

# **DOWNSTATE**

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### **ASSISTANT PROFESSOR -**PLANT DISEASE PHYSIOLOGIST

Assistant professor in plant pathology (70 percent research, 30 percent teaching) to develop a vigorous program on the physiology and biochemistry of plant-pathogen interactions. Teaching responsibilities will include a graduate-level course in plant disease physiology. Available 1 March 1982. Apply before 15 January 1982 to: Dr. Arun K. Chatterjee, Search Committee, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506. Equal Opportunity/Affirma-(NW169)A tive Action Employer.

# **Biochemist**

# **Pharmacologist**

SUNY Stony Brook has a position open immediately for one Research Associate (postdoctoral). Projects involve the mechanistic studies of a new antitumor drug or the chemical carcinogenesis.

Send résumé and 3 recommendation letters to: Prof. Felicia Y. H. Wu, Dept of Pharmacological Sciences, HSC, SUNY Stony Brook, Stony Brook, NY 11794. SUNY Stony Brook is an equal opportunity/ affirmative action employer (NW163)A AK 232B.

# SEMINARS and SYMPOSIA

# CALL FOR PAPERS for the

# FIRST ANNUAL SPINA BIFIDA ASSOCIATION OF AMERICA RESEARCH SYMPOSIUM

Papers should describe basic, clinical, or applied research projects dealing with causes, treatments, methods of detection or prevention of spina bifida hydrocephalus and related conditions. Deadline for abstracts is 1 February 1982. The meeting will be held in Dallas, Texas, on 23 April 1981. Contact: Dr. C. J. Sherry, Biology Department, Texas A & M University, College Station, Texas 77801.

(NW161)M

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£10 including Lunch, Tea & Coffee

For further details and application form please contact: Dr M J Daly, Medical Division, Glaxo Group Research Ltd.,

Ware, Herts SG12 0DJ. Telephone No. 0920 3232.

(060)M

# EMBO SENIOR FELLOWSHIPS

During 1982 the European Molecular Biology Organization will award a limited number of Senior Fellowships to exceptionally qualified molecular biologists from Europe and overseas wishing to spend a period of 6 or more months in laboratories in the countries listed below. These fellowships cannot however be awarded for exchanges between laboratories in the same country. To be eligible to compete for these fellowships a candidate must have at least 8 years post-doctoral research experience and have made distinguished contributions to molecular biology or related fields of science.

The fellowships will comprise a travel grant (tourist class air fare) for the fellow and his dependants and an appropriate stipend. Although initially awarded for not less than 6 months and no more than 12 months, the fellowships may, subject to review by the EMBO Fund Committee, be renewed for a further period.

Further information concerning the fellowships and stipends as well as application forms may be obtained from Dr. John Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 6900 Heidelberg, Federal Republic of Germany.

Completed applications recieved before February 19, 1982 will be reviewed by the Fund Committee on April 30, 1982 and any awards made can be activated immediately.

The Host laboratory must be one of the following countries; Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom. (W534)E

# IMPERIAL COLLEGE DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE POST-DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a threeyear post-doctoral fellowship to study the role of chemical additives to alleviate slagging in coal-fired boilers. The project is supported by the SERC and Babcock Pwer Ltd under the co-operative award scheme.

The aim of the investigation is to eludicate the role played by trace quantities of transition metal compounds in alleviating the slagging and thus improving the thermal efficiency of coal-fired boilers.

Applicants should have a first degree in chemistry, physics or an applied science. Industrial experience in coal combustion or research experience in the fields of electron microscopy, X-ray diffraction or high-temperature chemistry is desirable though not essential.

Salary according to age and experience, is on the scale £6,880 — £7,700, plus London Allowance of £967.

Applications, including the names of two referees, should be sent to Dr J Williamson, Department of Metallurgy and Materials Science, Imperial College, London SW7 2BP, by 2nd January, 1982. (056)E

# ISTITUTO NAZIONALE DI FISICA NUCLEARE (INFN) TWO POST-DOCTORAL FELLOWSHIPS IN THEORETICAL NUCLEAR AND PARTICLE PHYSICS

For one year, starting September 1982, for non Italian citizens. The chosen applicants can pursue their research at any of the following Laboratories and Sections of INFN:

National Laboratories of Frascati (Rome); National Laboratories of Leganro (Padua); National Southern Laboratory (Catania); INFN Section of Turin; INFN Section of Milan; INFN Section of Milan; INFN Section of Padua; INFN Section of Trieste; INFN Section of Bologna; INFN Section of Florence; INFN Section of Pisa; INFN Section of Rome; INFN Section of Rome; INFN Section of Pisa; INFN Section of Catania; INFN Section of Catania; INFN Section of Sanità (Rome).

The salary will be approximately 800 US dolars per month for twelve months.

Send applications and requests for further information to: Prof Dr Antonino Zichichi, Presidente, National Institute of Nuclear Physics (INFN) Piazza dei Caprettari, 70 00186 Rome — Italy.

The application deadline is February 15, 1982. (W539)E

# LINCOLN COLLEGE, Oxford

# EPA CEPHALOSPORIN JUNIOR RESEARCH FELLOWSHIP IN MEDICAL, BIOLOGICAL OR CHEMICAL SCIENCES

The College invites applications from graduates, of either sex, under 28 years of age on 1st October, 1982, for a Junior Research Fellowship in Medical, Biological or Chemical Sciences, tenable for three years from October, 1982; applications from older candidates will be entertained in special circumstances only.

Further particulars may be obtained from the Rector, Lincoln College, Oxford OX1 3DR, to whom applications should be submitted by 14th January, 1982. (013)E

# UNIVERSITY OF DUNDEE DEPARTMENT OF BIOCHEMISTRY

Applications are invited from Honours Graduates in Biochemistry for a RESEARCH ASSISTANTSHIP

funded for 3 years by the MRC. The successful applicant will work with Dr D A Stansfield on an investigation of the guanine-nucleotide regulation of LCG-sensitive luteal adenylate cyclase. Further details of the post are available from Dr Stansfield at the Department of Biochemistry, the University, Dundee.

Applications (3 copies) naming 2 referees should be sent as soon as possible to Mr K M Cocker, The University, Dundee DD1 4HN. Please quote Ref: EST/61/81J.

(073)P

### CONFERENCES and COURSES

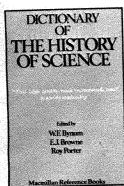
# EMBO COURSE IN ELECTRON MICROSCOPE IMAGE ANALYSIS European Molecular Biology Laboratory

The course, to be held from April 21st \_ 28th, 1982, is aimed at students who already have some background in biological electron microscopy or structural studies and who wish to learn the available techniques of computer image analysis. The following topics will be covered.

Methods for reducing specimen damage. 3-D reconstruction of periodic objects. Correlation methods for averaging.

Applicants should write to the organisers before 15th February 1982, giving the relevant details of their scientific background and their reasons for wishing to attend. No fee will be charged but the number of students will be limited to 20. Organisers: K. R. Leonard, J. Dubochet and T. Pitt.

EMBL, Postfach 10.2209, 69 Heidelberg, Germany. (W535)C



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Substantial concepts such as Evolution and Light are given generous entries, while more specialized subareas like Neo-Darwinism and Light Velocity are allocated smaller amounts of space. All are cross-referenced back and forth.

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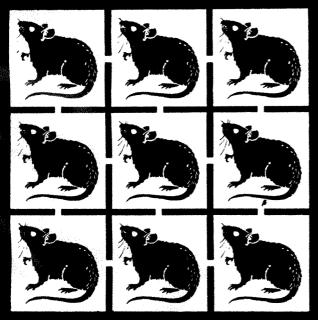
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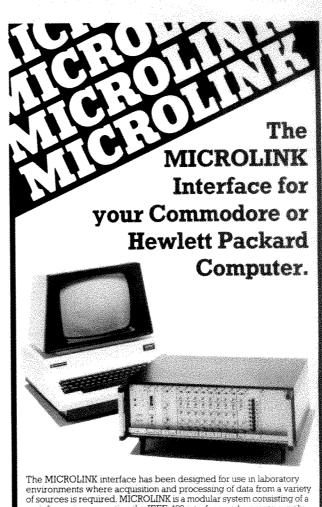
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Vol. 294 No. 5843 24/31 December 1981

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# CORRESPONDEN

Moscow plea/Molecular electronics/ Origin of life/Militant librarianship

# **NEWS AND VIEWS**

Lymphokines on the move (V Paetkau) A new view of supernova remnants in the Magellanic Clouds (D Clark) Are stress fibres contractile? (K Burridge) Order or disorder in the structure of glass? (S R Elliott) Calcium and calmodulin in Kyoto (R S Adelstein)

Environmental nitrosamines and cancer (V M Craddock)

Movement in membranes (A G Lee) The gassiest comet? (D W Hughes)

# **BOOK REVIEWS**

Charles Darwin: A Man of Enlarged Curiosity (by P Brent) A J Cain; Dynamics of the Upper Atmosphere (by S Kato) R Hide; Petroleum Geology of the Continental Shelf of North-West Europe, 1981 (L V Illing and G D Hobson, eds) J R V Brooks; Molecular and Cellular Biology of the Eye Lens (H Bloemendal, ed.) Christine Slingsby; Ecological Biogeography of Australia (A Keast, ed.) Peter D Moore; Biology of Conidial Fungi (G T Cole and B Kendrick, eds) C T Ingold; Conception in the Human Female (by R G Edwards) R V Short 781

Volcanic activity of "Anak Krakatau" photographed in 1979. A new interpretation of the Krakatoa eruption of 1883 is presented on page 699

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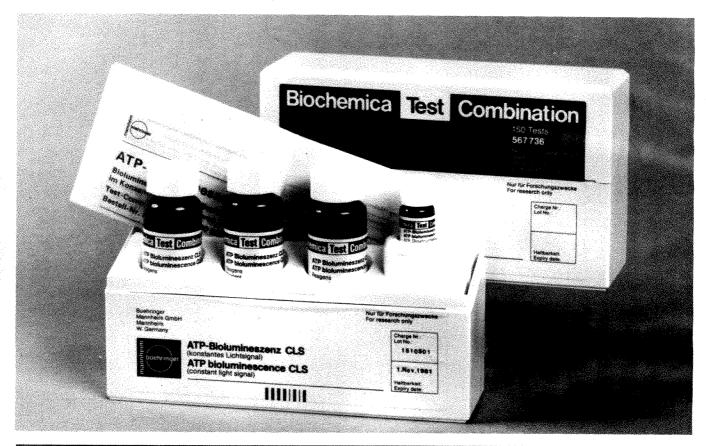
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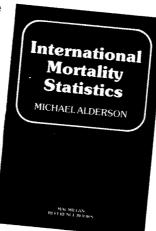
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Card. Enquiry on Reader 7

# nature

24/31 December 1981

# Poland, Christmas, Sakharov et al.

The scientific community cannot complain that some of its members live in states whose laws are illiberal, but should it acquiesce in the arbitrary loss of jobs?

The coincidence of recent events in Eastern Europe with the Christian celebration of the ancient festival of the winter solstice is not accidental. If, in Poland, the tide of social change that has occupied the past eighteen months is to be turned back by the threat of force, when better than this time of the year, when life is hard and food even more scarce than usual? But this is also the time when the despair of the despairing is heightened by the sense that winter's grasp has yet fully to close. This may help explain why a group of scientists in the Soviet Union has written to Nature to complain at their government's emigration policy (see page 688). Their letter is unlikely to make life easier for them, at least in the immediate future. The authors are nevertheless aware of what the consequences may be. That they have written their letter is a sign that they consider more conventional means of pressing their case have run into the sand. Will what they have to say be heard against the din of the forced jollity of the days ahead?

The authors of the letter appeal to the international scientific community, of which they wish to be a part. What response is appropriate? This question has much in common with those which have exercised scientific academies in the past several years. A few simple truths stand out. No part of the scientific community is competent to complain at the legal practices of individual governments, however illiberal or unjust. If, for example, it is still the practice somewhere to punish a thief by cutting off his right hand, that is no part of the scientific community's business. Similarly, scientists used to Western ways, and in particular to the knowledge that people's freedom to live and work elsewhere is constrained only by the willingness of host countries to receive them, have no right as scientists to complain that Soviet law gives the administration the right to withhold exit visas. It is true that the scientific community is impoverished - and the Soviet Union made to look foolish - when Soviet scientists are prevented from attending international conferences, but even that is not an occasion for public protest but a matter for diplomacy among the scientific academies (which, nevertheless, should be more energetic in this cause).

The disturbing feature of this week's letter from Moscow is that it suggests that individuals scientists in the Soviet Union may be victimized as scientists while acting within the law. It is common ground that to apply for an exit visa is not a crime. The fact that Dikii et al. are Jewish is (or should be) irrelevant, embarrassing though the flood of would-be emigrants to Israel has been for the Soviet government in the past decade. And their plight is by no means novel. Many Soviet citizens who have become in some sense awkward customers have found the loss of their jobs to be the first sign of having given offence and often a prelude to more serious trouble. Moreover, the loss of a job is not exclusively an accident befalling those who wish to go to Israel. There is at least one unpublished case of a graduate student who has been dismissed from the institute at which he was hoping to complete the work for his doctoral thesis on the heels of his application to join his wife in Western Europe. On the face of things, however, even this accumulating evidence of illiberality is not a sufficient cause for the scientific community to take up cudgels. If the convention is being established that those who legally fail to toe the conventional line will in the first instance lose their jobs, and if the sanction applies to all and sundry, surely the international scientific community has no special status in the matter? That is the

simplest (and most convenient) conclusion. It is, however, wrong.

First, it is more than probable that the convention that those who make trouble should lose their jobs will bear with especial severity on scientists in the Soviet Union. Continuity, as Dikii et al. proclaim, is crucial, especially early in a career; other kinds of professional people are usually (but not always) less vulnerable. Second, the importance (numerically and prestigiously) attached in the Soviet Union to professional work in science and technology is almost a guarantee that technical people will be prominent among the casualties of illiberality. Third, if scientists lose not only their jobs but access to scientific publication and are at the same time stripped of their degrees — a fanciful process in itself — the record of research is falsified. These are in themselves sufficient reasons why the scientific community elsewhere should take more than a passing interest in what is happening in the Soviet Union. But the most serious cause for alarm is the now well-understood procedure for arranging that those who figuratively blot their copybooks will then lose their jobs. Most Soviet laboratories have two directors, the better known of whom is a distinguished scientist. His partner is more directly concerned with political matters, including the hiring and firing of people. One of the unacceptable ironies of these arrangements is that the scientific heads of important institutions (who seem to have no difficulty with short-stay exit visas) are able to boast of the bright young people who work for them but to deny responsibility for those who have lost their jobs, to become dependent on the charity of friends and former colleagues. Such detachment (even if occasionally mirrored in the attitudes of the heads of laboratories in the West) is unfortunately not merely offensive but potentially destructive of the scientific enterprise. If bright young people are arbitrarily denied the chance to work the stint of which they are capable, and if the numbers are as substantial as those now accumulating in the Soviet Union, the enterprise will be undermined, not just impoverished. Those who enjoy the privilege of being directors of important Soviet institutes should be left in no doubt of how, by their indifference to or neglect of what is happening to the people in their charge, the consequence will be not merely to impoverish but to corrupt the scientific enterprise.

None of this will help Dikii et al. What should they do? Go on hunger-strike, like Andrei and Yelena Sakharov? For, contrary to expectations (see Nature 26 November, p.295) that ploy has worked. Dr Sakharov's putative daughter-in-law, Liza Alexeveva, has now joined his stepson in the United States. Mercifully, the Sakharovs are able to eat again (but they must please remember that they cannot keep on doing this, as they get older, with like impunity). Time will no doubt tell on what basis Liza Alexeyeva's departure from her homeland and the fasting Sakharovs was arranged. The Sakharovs probably compromised very little. But it is also plain that their despairing wager of their lives has been an uncharacteristically narrow venture. Their daughter-in-law has got away. The graduate student who impetuously married his legal wife is still stuck without a job somewhere in the Soviet Union. Should he ask his parents to go on hunger-strike? Both he and they, of course, know that that would carry relatively little weight. They may all think it a pity that the Sakharovs have spent their great influence on the solution of one person's problem when they might have been able to contribute to

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a much more general resolution of a general important problem — even if one that is not directly the concern of the scientific community. That even brave people such as the Sakharovs might have been able to accomplish a step towards that goal is naturally a conjecture — and at times like the winter solstice, even people like them will be forgiven for putting Christmassy personal considerations before grand and perhaps unattainable causes.

None of this will much help the scientific academies or the individual members of the scientific community to know more clearly where they should stand on the events of the past few weeks. On the most immediate issues, they have no competence to make pronouncements of a scientific character even if they may choose to be vociferous in other roles. Precisely how the residue of what the Poles used to call Solidarity should be succoured is beyond their competence as scientists (although some, as scientists, may in future think differently of the problems of European defence). The only legitimate opening in this haunting argument for the scientific community is that represented by the hypocrisy of the institute directors from the East, who pretend that all the dreadful things that happen to those who used to be their young men and women are outside their competence. That defence can no longer wash - and it would be better for all concerned if the Soviet authorities would make (as would be their right) a simple rule that those who have benefited from the Soviet educational system must work their passage towards emigration by some system of indenture - so many years of public service for such and such a qualification would at least let people know where they stand, and would be consistent with the principle that sovereign states should be free to decide how to run their affairs. The growing practice of depriving skilled people as a kind of punishment of the right to practise their skills is a loss of more than a Soviet resource, and is offensive. Indeed, it is a disgrace.

# British academic agony

The British government has relaxed pressure on its universities, which may fail to take advantage.

The British government has now agreed to pay the second instalment of the cost of reorganizing and running down the British university system, decreed by the then Secretary of State for Education and Science just over a year ago. During the present academic year (which ends on 31 July 1982), the University Grants Committee has been able to set aside a total of £20 million to help with the cost of paying off redundant academics. In the following year, there will be a further £50 million to spend on measures of reorganization in the university system, but the government bravely says that it hopes not all of that will have to be spent on putting academics out to grass. If the Committee of Vice-Chancellors is to be taken at its first word on the subject (which would be imprudent), the settlement for next year will be totally inadequate to pension off university teachers whose tenure of their jobs cannot be sustained. In reality, of course, the crunch will come in the succeeding financial year, when the universities will have reduced their student numbers to those required of them by the University Grants Committee, and when their urgent need will be to trim their sails for the long haul that will by then seem to lie ahead. But the government has promised to provide a still unspecified amount of money to cover the costs of that year's mayhem; is it hoping that in the meantime (during 1982-83) the universities will have the wit to think of doing novel things, things they have not thought of yet?

The difficulty in understanding the conflict between the British government and the British university system is that it is not so much a conflict between an irresistible projectile and an immovable object as between blancmange and jelly (or jello). The government has discovered, late in the day, that the cost of firing tenured academics is not negligible, but dare not say so. The universities, on the other hand, are so frightened and affronted by what has happened to them in the past year, in particular by the discovery that they have fewer friends than students, that they

behave as if they have no choice but to pass on to their paymasters the information that they have gathered from the Association of University Teachers, the mild-mannered labour union capable nevertheless of making a lot of noise, that the cost of any change is bound to be greater than the cost of comfortable no-change. The time has come to ask whether even that seemingly prudent course can be wise.

Like the constituents of other university systems, British universities are naturally jealous of their autonomy. Their jealousy is, however, undimmed even while (as now) they are learning to live with instructions from the University Grants Committee that the numbers of students recruited in various fields should be determined in advance. The conventional statement of academic freedom is that self-governing universities should be free to decide for themselves who should teach what to whom. The difficulty, in present circumstances, is that the who is defined by the survivors now in post, that the what is largely determined by extension and that the to whom, the students, make their own numbers through the medium of the Universities Central Council on Admissions, falling so to speak where the academics lie. The new restrictions on student numbers rob the university system of a further degree of freedom, a loss which collectively the universities have not yet chosen to complain about. They should.

Individually if not collectively, they should also give some serious thought to what the more distant future holds. Although the government has been able to put some kind of limit on the cost of other kinds of higher education than those provided by universities, the lack of coherence between the universities and the polytechnics persists as a weakness of both systems. The universities, the more senior of the partners, should acknowledge that it is, in these hard times, for them to bridge the gap. While they are about it, they should also give more serious thought than has been their habit to the needs of their students and of their students' potential employers. British students are too highly specialized; British graduates are too uniformly academic. Not all but most universities should change their habits of selection and their ways of teaching so as to cater for a greater diversity of people and of interests. Over the past twenty years, British universities (singly and collectively) have resisted the notion that students are not all ideally the same, and that students' ambitions sometimes encompass secularly non-academic goals. That is obscurantist.

The plain truth about the distant future is that British universities are at present organized in such a way that they will all (except Oxbridge) sink together unless they have the courage to acknowledge that survival requires that they should exercise the autonomy of which they boast. Several corollaries arise. Academics should not always be paid the same, under "nationally negotiated agreements"; instead, universities should be free to pay their people what they can afford (and earn). Tenure should be limited, taking some account of the need to preserve people's freedom to write (and even to say) what they think fit, but acknowledging that universities cannot make lifelong contracts with their employees while living from hand to mouth themselves. The result of such an erosion of the common academic sense of security would send the better academics flocking to two contrasting kinds of places - secure universities valued for their sheer academic excellence and much more specialized places valued for their special skills at teaching students what much of the real world expects of them. The result, of course, would be more diversity among institutions of higher education. Would that necessarily be disastrous? The system as it is is calamitously

So how to get from here to there? The interpretation of the British government's budget restrictions by the University Grants Committee will, if unchanged, have an ossifying effect. Universities individually will preserve their present character but will shrink. Strange though it may seem, the universities probably find that dismal prospect preferable to the better alternative — a framework in which they would be enabled but also compelled to find for themselves a niche in the market in higher education.

# Balance of US energy research attacked

# Advisory group gives comfort to Reagan's foes

Washington

A top level advisory committee to the US Department of Energy (DoE) has told the Reagan Administration politely but firmly that it is not making the best use of its resources for energy research. In particular, the committee says that there is insufficient support for conservation technologies and that too much is being spent on nuclear energy.

The report was published shortly before President Reagan's announcement last week that he is soon to submit plans to Congress for dismantling DoE. He said that the purpose of the move is to create "a strong federal effort in basic research in energy that avoids excessive regulation".

The centrepiece of the reorganization is a new Energy Research and Technology Administration within the Department of Commerce. The plan is expected to meet fierce opposition in Congress, particularly from those who favour an independent energy research agency on the grounds that close links with the Commerce Department would reinforce the alleged pro-nuclear bias.

The criticisms of present energy research policies came from DoE's Energy Research Advisory Board (ERAB), an independent, non-partisan group. The chairman of the board is Mr Louis H. Roddis Jr, previously president of Consolidated Edison of New York, and the report was prepared at short notice at the request of DoE by a panel, led by Dr John S. Foster, vice-president for science and technology at TRW Inc., comprising all ERAB members and several outside consultants.

Objections to the Reagan plan are already coming from another direction, namely congressmen unhappy at the proposal that the nuclear weapons research carried out at the nation's weapons laboratories should be among the activities transferred to the Department of Commerce. Speaking at the National Press Club in Washington last week, commerce secretary S. Malcolm Balrige said that his department's experience with other research agencies, such as the National Oceanic and Atmospheric Administration, made it a natural place for locating energy research. He also admitted that the Commerce Department's responsibility to assist in increasing exports could logically include the international promotion of American nuclear technology.

The ERAB report takes as its frame of reference the policy guidelines laid down

by the Reagan Administration, thereby insulating itself from the criticism that it is making a political attack on Mr Reagan's policies. Although in principle enthusiastic about the new policy directions, the ERAB committee is concerned about how decisions, such as the cutting back of research funds for conservation and solar technology, have turned out in practice. And the report champions higher energy prices as the best way of encouraging efficient use of energy and utilization of new energy sources.

All 20 members of the committee agreed on the need to emphasize new energy technologies where research has already reached the stage at which the results could, if desired, be directly taken over by the private sector, and should otherwise be dropped if this desire does not exist. For example, the report recommends the elimination of support for research into small-scale hydropower and magnetohydrodynamics (decisions already made by the Reagan Administration), and a sharp reduction in support for research on electric vehicles.

Little change is advocated for most of

the basic sciences whose budgets are the current responsibility of the department, including high energy physics and nuclear physics, and which would be incorporated directly into the new agency. And the board favours greater support for efforts aimed at improving the quality and quantity of scientific personnel at US universities in energy-related areas, described as an area "appropriate for federal concern" but currently receiving only \$10.6 million a year.

The most controversial parts of the report are those which address directly the government's role in supporting research into the generation of electricity through nuclear power. The ERAB report describes the Clinch River fast breeder reactor as being "not an urgent priority", recommending that, under current budget constraints, such a demonstration project should be postponed. Four members of the advisory board, however, dissent from this opinion on the grounds that not proceeding with the construction phase of the fast reactor would mean writing off the \$1 billion that has been invested so far, and

# What case for building Clinch River?

Washington

Following Congress's decision last month to give the go-ahead for the construction of the liquid metal fast breeder reactor at Clinch River in Tennessee, the focus of the controversy surrounding the project has shifted back to the Nuclear Regulatory Commission (NRC), which would have to issue a permit allowing the construction to proceed.

Last week, the five members of the commission agreed to consider a request from the Department of Energy that it be given "emergency" exemption from conventional licensing procedures. According to Energy Secretary James Edwards, such an exemption is necessary to avoid the "undue hardship" which, he told NRC, would result from further hold-up in the long-delayed construction plans.

Critics of the reactor, however, claim that the Department of Energy is trying to manoeuvre NRC into providing a provisional construction licence as soon as possible so as to pre-empt further attempts by Congress to terminate the project. The critics claim that their case is substantiated by an internal Department of Energy memorandum, released last week by the Natural Resources Defense Council (NRDC), in which Under-Secretary of Energy Guy Fiske suggests that the request for an emergency exemption from NRC be withheld until the 1982 appropriation bill is passed - an amendment could

have been added forbidding the Department of Energy from making such a request — and that the Council on Environment Quality be asked about securing "strong support" for an internal environmental report establishing the "negligible environmental impact" of the construction of the reactor.

NRDC attorney Barbara Finamore said that the memo "reveals a calculated effort by the Department of Energy to undermine the integrity of the NRC and its licensing process". Supporters of the fast reactor, that the Clinch River project is needed to demonstrate this technology in the US licensing and safety environment.

Although the construction of the Clinch River reactor has been approved by Congress, its completion is not certain. It is generally accepted in Washington that the main reason for Congress's approval was political—the project is strongly backed by Tennessee Senator Howard Baker Jr, the leader of the Senate.

One possible outcome is that preliminary construction work will be carried out, partly to satisfy the pro-nuclear supporters in Congress to whom the fast reactor has become an important symbol, but that eventually even the utility companies which now back the project will agree that it is inappropriate to continue, and the Clinch River reactor will be abandoned on the grounds of its inefficiency and antiquity.

David Dickson

dissipate the "hard-won technology capability" already developed in the United States. The dissenters were ERAB chairman Louis Roddis, William S. Lee, president of Duke Power Company, Roland W. Schmitt, vice-president for corporate research at General Electric and John W. Simpson, a consultant previously with Westinghouse Electric. Described by critics as the "electric mafia" on ERAB, these same four are said to have been successful in toning down parts of the report which criticized the emphasis being given by the Reagan Administration to nuclear research as disproportionate to nuclear's role as an energy source.

The final report states merely that 'although no correct balance among energy forms and resources can be defined a priori, research and development for electric supply technology is receiving a larger proportion of funding than the present and projected share of electricity in our national energy supplies". In addition to recommending that the Clinch River fast reactor should not be built, the panel suggests that high priority be given to research into nuclear waste disposal and into conventional light-water reactors, and a "little less" funding into both breeder reactor fuel cycle research and magnetic fusion. **David Dickson** 

#### Data falsification

#### Harvard acts

Washington

Harvard Medical School announced last week that it was setting up a special committee of both faculty members and outside academics to recommend how the school should deal with future cases in which research workers are accused of producing falsified research data.

This announcement follows the resignation of a member of the medical school research staff, Dr John R. Darsee, who admitted that he had fabricated research data during an experiment last year which involved efforts to limit the damage caused by heart attacks in animals.

Dr Darsee also resigned his post as a research fellow at the Brigham and Women's Hospital in Boston. Before his resignation, according to reported comments from other members of the hospital staff, he had been under consideration to head a hospital laboratory.

As a result of allegations about Dr Darsee's work made last year by other research workers at the medical school, an investigation was carried out, in the course of which Dr Darsee admitted that he had falsified the research results in question.

A statement issued by the dean of the medical school, Dr Daniel C. Tosteson, last week stated that "none of the work which could not be verified has been presented to the scientific community". Earlier this year, Dr Darsee's supervisor, Dr Eugene Braunwald, decided that all

#### Christmas present for Heidelberg laboratory

The European Molecular Biology Laboratory at Heidelberg has half the Christmas present it was promised a few months ago: a liquid-helium temperature transmission electron microscope lens from Siemens AG, Munich, but not the pictures that should follow from it.

Dr Arthur Jones, head of the electron microscopy group at the laboratory, had hoped the system would have been operational by Christmas. But the lens — only the second commercial such lens in existence (the other went to Berlin) — arrived only a week ago. The first pictures, probably of hydrated crystalline specimens at 4 K, are likely to follow in January. Biological specimens should be in view in the spring.

Excitement is intense in the group about what they see as the most important development in electron microscopy for more than a decade. The Siemens group, headed by Dr I. Dietrich, appears to have shown that specimen damage by the electron beam — which limits the available resolution of a microscope on sensitive, nonperiodic biological specimens — may be reduced by orders of magnitude at 4 K.

And at the same time, Dr Jacques Dubochet, in charge of specimen preparation, claims now to produce hydrated biological specimens containing vitrified — non-crystalline — ice. (The formation of crystals would create artefacts and interfere with the imaging of specimens.)

At first, however, the resolution may be no better — or even worse — than with conventional electron microscopy, because the contrast available in hydrated specimens is much less than that possible with negative staining. The reduced beam damage, however, should compensate for this by allowing greater illumination, with the result that the microscope might reveal structures without the artefacts of the conventional stains. Ultimately the use of samples labelled with heavy atoms could make a greater, artefact-free resolution available says Dubochet.

After this "cryo-TEM" will come the scanning version, the "cryo-STEM". The first electron beam down the cryo-STEM is expected by mid-year, with the first images shortly after.

Robert Walgate

abstracts of Dr Darsee's work should be withheld from presentation at the annual meeting of the American Heart Association.

The National Institutes of Health (NIH) were also notified of the alleged falsification of data, since Dr Darsee was working on NIH-sponsored research and was in receipt of an NIH research fellowship, which he has since resigned together with his academic posts.

The advisory panel set up by Dean Tosteson is being chaired by Dr Richard Ross, dean of Johns Hopkins School of Medicine. According to last week's statement, the panel has been asked "to review the case in question, and to indicate whether or not any additional measures should be undertaken, and to recommend procedures for dealing with episodes of this kind in the future".

The committee has already started work, and its report is expected early in 1982.

**David Dickson** 

#### Molecular biology

# Limited progress

When the director-designate of the European Molecular Biology Laboratory (EMBL) in Heidelberg takes over from Sir John Kendrew in April, he will not find much room for movement in the budget. Last June, Sir John asked the ten-nation council for DM 32 million (£7.5 million) for 1982. In the event the council has now agreed to spend DM 30.2 million, roughly a 10 per cent increase on 1981 compared with a German inflation rate of about 6 per cent.

That allows for a small increase in staff this year — some 20–25 of whom 8–10 will be scientists, according to finance director Eckhart Weis. Thus the laboratory will edge ahead of local inflation, but will come nowhere near the "indicative scheme" prepared in 1980 which foresaw a budget increase of 20 per cent and a staff of 265 rather than the 220 now employed. The directorate, for "scientific reasons", did not fill the 265 posts which were available in 1980, and since then the recession has meant that the council would not agree to the budget which would be required to reoffer them.

Meanwhile, the new EMBL director, Professor Lennard Philipson of the University of Uppsala, refuses to define his policy for the laboratory until after he is in post. But he has been taking regular soundings, and, it seems, gaining everyone's confidence. He is said to recognize the important and unusual role that physicists play at the laboratory and the significance of the EMBL outstations at DESY, Hamburg (a synchrotron radiation source which has a long queue of applicants for beam time) and the Institut Laue Langevin, Grenoble (a neutron source).

It seems likely that the new regime will see a greater integration of the work at Heidelberg, and between Heidelberg and the outstations, with the selection of two or three principal areas of biology (cell membranes, for example) as broad foci of interest. But there will be nothing so blockbusting as an attack on the whole human chromosome — "that's factory work" said one senior EMBL scientist. On the

instrumentation side, certain techniques, such as gel electrophoresis, which are central to the new methods of molecular biology, may be singled out for increased attention; but Philipson is also said to have shown his support for the great efforts being made at Heidelberg to improve electron microscopy through cooling the microscope and specimen (see accompanying box).

At present Sir John Kendrew is still firmly in charge. He foresees an increase in the number of permanent scientific appointments (still less than a handful) at the laboratory in the next couple of years, as a batch of the early short-term contracts come to an end. Philipson, however, has made no commitments. As for Sir John himself, he feels that the seven years and three months for which he will have held the directorship is quite long enough.

Robert Walgate

#### Polish crisis

#### Science in limbo

The "state of war" proclaimed by General Jaruzelski's "Military Council for National Salvation" has brought all academic life in Poland to a standstill. The universities are closed as are several institutes of the academy of sciences. Scores of scientists and scholars have been arrested although some, such as the president of the academy, Dr Aleksander Gieyszter, have since been released. Others escaped arrest only by chance, being away from home when the police came, and are now in hiding. Solidarity organizers in the learned professions and activists from the Independent Students' Association have been interned. The Minister of Science, Higher Education and Technology, Dr Jerzy Nawrocki, is reported to have resigned.

This clamp-down on intellectuals is in sharp contrast with Jaruzelski's previous attitude. In a recent interview, Dr Leonard Sesnewski, one of the vice-presidents of the academy, said that Jaruzelski had always expressed warm feelings for scientists and that, on taking office as prime minister last February, he had immediately visited the academy to ask for their help in getting Poland out of its economic crisis.

Nevertheless, the growing drive for academic autonomy must have caused a certain friction between the general and the academy. At present, the academic secretary of the academy holds quasiministerial rank, and is responsible to the prime minister. In recent months, however, there has been considerable pressure from academy members and employees to incorporate into the proposed new bill on the academy clauses that would terminate this structure and make the academic secretary responsible only to his fellow-academicians. There has also been a drive - reflected in the Solidarity Congress resolution on learning and culture — to break down the barriers between the universities and the academy, so that academy employees could deliver lectures to undergraduates. This trend may have seemed dangerous to the authorities; a number of scholars, expelled or excluded from teaching posts for their political views over the past few years, have found a safe haven in academy research posts insulated from teaching.

For the time being, the concern of scholars abroad is focused on the plight of their Polish colleagues interned or in hiding. Professor Olof Tandberg and his colleagues from the Swedish Academy of Sciences (which traditionally has strong links with Poland) and several members of the Norwegian Academy have launched an appeal for financial support for the families of interned academics. Professor Tandberg has also received an appeal from a group of academy scholars in hiding asking for food, blankets and other essential supplies, including blood plasma. He has called on all other academies throughout the world to join the Norwegian and Swedish academics in this Vera Rich work.

#### UK agricultural research

# Endangered duo

Two British agricultural research institutes, the Long Ashton Research Station in Bristol and the Animal Breeding Research Organisation in Edinburgh, could be drastically cut or even closed if an economy proposal by the Agricultural Research Council is approved at its next meeting in February. Under threat are all research programmes at the Edinburgh institute except work on fundamental animal genetics, and the Pomology and Food and Beverages Division at Long Ashton.

The council (ARC) hopes the closures will save about £3 million in annual expenditure by 1983-84. (The budget for 1981-82 is £86 million.) According to Dr Ralph Riley, the secretary of ARC, the savings are needed both as a hedge against future government cuts and to allow greater flexibility in supporting research of high priority. The council's research programme has already suffered, he says, from a budget that has not kept up with inflation. Non-payroll expenditure has already been pared to the bone. But precisely how much of the £3 million saved will be available for starting new research projects after cuts in income are taken into account remains uncertain.

If the proposal is approved, the Long Ashton Research Station will be reduced to about one half of its present strength, with the loss of about 100 jobs. The Animal Breeding Research Organisation, however, will probably be closed and the research on animal genetics, which accounts for about one-fifth of the organization's activity, moved elsewhere.

ARC has made the proposal without

consulting the staff or directors of the institutes concerned, who first learnt of it only last week. The idea, according to Dr Riley, was for ARC to propose firm suggestions for savings and then to give those concerned two months in which to make a case before a final decision. The way in which the proposal was arrived at remains something of a mystery, but the main criterion seems to have been an assessment of priorities based on recent reviews of the work of all the council's institutes. Areas in which the council is likely to want to spend more money include fundamental genetics and biotechnology.

If approved, the proposal could have implications for the Ministry of Agriculture, Fisheries and Food, which spends about £44 million a year in ARC institutes on commissioned research. The ministry is optimistic that the work it commissions in the institutes under threat could be transferred to other institutes. Dr Riley, however, believes that this will be possible for most commissioned research but that there may be some areas where the ministry will have to look elsewhere. Judy Redfearn

#### High-energy physics

#### LEP marches on

The large electron-positron ring is on the move — in more than one sense. LEP will be the next big accelerator at CERN, the European Centre for Nuclear Physics, Geneva, and last week CERN member states finally agreed on the budget under which it will be built. Thus construction can begin as soon as local environmental approval is granted — by January or February, it is hoped. The second move is physical: LEP is to be built in a significantly new position, on a slope, which allows it to slide out of some tricky geological and political problems.

The budget agreed is sufficient, says CERN director-general Professor Herwig Schopper, to build LEP on the schedule foreseen a few months ago: fast enough to give colliding electron and positron beams of sufficiently high energy to provide physicists with copious neutral intermediate vector bosons by the end of 1987.

LEP is expected to cost SF910 million (about £263 million) at 1981 prices, with a further SF40 million (making a total of £275 million) for the first experiments. The money will be drawn from a guaranteed CERN annual budget of SF617 million.

The only major argument at the council was over what inflation index to apply to the 1981 budget to scale it up to 1982 prices. The usual CERN formula, appropriately weighted for CERN's Swiss salaries and expenditure abroad, gave 5.7 per cent. The council quibbled, and agreed on 4.4 per cent, losing CERN over £2 million next year.

The new slope for LEP (it was to have been horizontal) will be about 1.5 per cent from the Jura Mountains in the north west, towards Geneva. This enables the machine

to be slipped away from the cavernous Jura limestone, and further into the stable sandstone of the valley, in which all previous CERN accelerators have been built.

The result is that only 3 km (as opposed to 8 km previously) of the 27 km tunnel for LEP will be in the unpredictable limestone. Moreover, at the Jura side the ring will be higher — about 140 metres below the surface — so that if any difficult waterfilled caves are encountered, it will be possible to deal with them from above.

A further — and far from negligible — benefit may prove to be political. There has previously been vociferous environmental opposition to LEP from the French side (the Jura; LEP straddles the French-Swiss border). This opposition rested in large part on the (distributed) effect that the limestone borings might have on the source and flow of the River Allondon, which supplies water to a number of villages. The source was to have been within the LEP ring; now it is outside, and where the ring passes under the river it is within stable sandstone.

LEP will be paid for out of the current budget only by running down certain existing facilities. The intersecting storage rings for protons and other nuclei will be closed at the end of 1983; and operations on the 600-MeV synchrocyclotron (CERN's first accelerator) will be reduced to the mainly Scandinavian Isotope Separator On-Line (ISOLDE), which performs unique experiments on shortlived muclei and, incidentally, keeps the Nordic countries happy. Robert Walgate

#### UK university funding

# No reprieve

The British government is sticking to its guns over cuts in university grants, indicating that universities have failed to convince the government that spreading the cuts over five instead of three years would cost less because compulsory redundancies, involving large compensation payments, would be avoided. Last week, Sir Keith Joseph, Secretary of State for Education and Science, reaffirmed his belief that the cost to the taxpayer will be less if the cuts are implemented quickly.

Hence, it is no surprise that the universities' recurrent grant for 1981-82 has been set broadly in line with the government's expenditure plans of last April. The grant at £995 million will be only £16 million more than the estimate, to account mainly for an inflation rate higher than expected. The research councils, however, may have come off slightly better than feared. At £478 million, the science vote for 1982-83 will be roughly in line with this year's figure, although the allowance for increases during the year is only 5.5 per cent.

Despite his rejection of the universities' argument, Sir Keith has nevertheless accepted that the cost of redundancies

cannot be met out of the universities' recurrent grant. But his allocation of £50 million in 1982-83 for restructuring the university system has already met with derision from the Committee of Vice-Chancellors and Principals which says that the sum is far too small. The vice-chancellors also fear that if the 5.5 per cent inflation allowance cannot be met then the number of redundancies will increase. A sum for restructuring in 1983-84 will be announced some time next year.

Sir Keith expects to reach a decision early in the new year on the scheme proposed by the vice-chancellors' committee for compensating redundant academics. Academics who take their cases to court, however, could be awarded considerably more than indicated under the scheme, making it almost impossible at present to estimate the total redundancy bill.

Advanced higher education has fared worse than any other sector of education under the government's cutbacks. The overall reduction in the education budget next year compared with this is one per cent, with further education for non-academic school leavers winning increased support. Clearly, the universities have lost out to the much stronger voice of the growing numbers of unemployed.

Judy Redfearn

#### Recombinant DNA research

# EEC safety dispute

Brussels

A deep division of opinion has become evident among the EEC's institutions over the need for strict legal controls to minimize the dangers of research using recombinant DNA techniques. By a narrow margin, the European Parliament's Committee on the Environment, Public Health and Consumer Protection came out against the EEC going than merely making recommendations on the registration of all relevant research. However, following a colloquy held in May (see Nature 21 May, p.181) the Economic and Social Committee (ESC) is strongly advocating that the European Community adopt a legally binding text enforcing tight safety controls.

Both opinions will be taken into acount by the ultimate decision-makers in the Committee of the Permanent Representatives to the EEC (Coreper) whose experts have been awaiting the views of the two consultative bodies.

The issue has been subject to an unusual amount of debate. In 1979 the European Commission itself proposed a legally binding directive along the lines demanded by ESC. This was then withdrawn and replaced by a set of recommendations to take account of evidence and scientific opinion which increasingly suggests that the dangers from bioengineering are less than were at first feared.

ESC thinks that the recommendations are too weak and that the reasons put forward to justify the original draft directive still hold good. It still considers that in the long term the unforeseeable and potentially serious consequences of recombinant DNA work require a "better safe than sorry approach", especially when pathogens are used as vectors or hosts. Also, the EEC has a responsibility to ensure that competition for commercially applicable research is not distorted by different rules on what can be done, and at what speed, in each member state.

The colloquy held by ESC to debate these points failed to budge the committee from its opinion, although many of the speakers there affirmed that the risks associated with genetic manipulation are small or negligible. The committee, however, remains convinced that transferring the techniques from laboratory to factory will not mean lower safety standards. Official guidelines would, therefore, be better than a system of self-regulation.

The report produced by Italian Euro-MP Domenico Ceravolo for the parliament echoes many of these concerns, but his resolution in favour of a directive was overturned in the committee vote by a majority of only one. In his report, Ceravolo attacks the European Commission's view by saying that even if a risk is only based on a hypothetical chain of events, this is no justification for thinking it any less valid or significant. And he argues further that the conjectural risks cannot be dismissed because no suitable criteria are available for assessing them.

Whether his arguments will win the day in the parliament's plenary session remains to be seen. A full vote was postponed at the last minute on 18 December but the vote is expected to go against Ceravolo. The liberals and conservatives, who form the majority in the house, support the Commission and feel that too much legislation will slow down the growth of Europe's biotechnology industry. The socialists and communists disagree and take their cue from the Italian left wing which sees EEC legislation as the best way of bringing Italian research under control.

Jasper Becker

#### Electronic publishing

# Journal plugs in

The British experiment to explore the feasibility of electronic scientific communication is well under way. The experimental electronic journal of the Universities of Loughborough and Birmingham, Computer-Human Factors, has received 16 papers in its first year — more than an earlier experiment in the United States received in its three-year life. Last week, the British Library, which is backing the experiment with £256,000, organized a demonstration for publishers,



journalists, editors and librarians.

The experiment is designed chiefly to identify the problems encountered by authors, editors, referees and readers in conducting editorial business on computers. Consideration will also be given to the possibility that electronic journals could fulfil the role of conventional journals. The experiment is also being used to investigate the role of computer networks for other less formal types of communication, such as newsletters, requests for comments on papers before submission to the journal, general communication between groups working on similar problems, the collaborative writing of papers and simply sending messages.

Members of the team working on the project say they are satisfied with the first year's results, ascribing their electronic journal's success in attracting papers to flexibility. In its purest form, an electronic journal would eliminate all paper; writing and editing would be done by means of VDUs (visual display units) and all transactions carried out over telecommunications links. Readers would also have access to the journal on their VDUs from a central computer memory.

Users of the journal preferring to see results on paper can get hard copy from printers at their terminals, and authors are also given flexibility by being allowed to submit papers either on-line or in the conventional way by posting typescripts to the editor. The editor can in-put perfect typescripts by optical character recognition but has to type in untidy ones on a word-processor. Of the 16 papers submitted so far, two have been on-line, and the rest came as typescript too untidy for optical character recognition.

The project, under editor Professor B. Shackel and his assistant Dr David Pullinger, is based at the University of Technology, Loughborough, and the central computer facility is provided by the University of Birmingham under the direction of Professor P. Jarratt. The 50 participants in the project, from universities throughout Britain, make up the journal's contributors and its only readers. Contributors are allowed to submit papers to conventional journals three months after submission to the electronic journal.

Although that option undermines the value of the electronic journal, its absence in the earlier United States experiment is thought to have dissuaded many potential contributors.

With two more years to run, the project is still at an early stage and the team is reluctant to draw many conclusions. Questions to be addressed, however, include the extent to which users can manage without paper, whether electronic journals could publish faster than conventional journals, the suitability of publishing papers and letters as soon as they are accepted rather than in batches as "issues" and alternative methods of refereeing.

Cost comparisons between electronic and conventional journals will be particularly difficult to assess. Capital cost could be minimized by using equipment initially acquired for other purposes, but running costs — chiefly the cost of using the telephone — will fall not only on the "publisher" but also heavily on users. One particular headache is how to compare the cost of reading time for conventional and electronic journals.

Even if this latest experiment demonstrates that electronic journals are feasible, the day when they become a practical reality in major subject areas is a long way off. The electronic journal, if it arrives, is likely to creep in gradually. Conventional journals, for example, may introduce new technology giving authors and readers the option of on-line access. But the problems of going entirely electronic are too formidable to be contemplated seriously for a few years yet.

Judy Redfearn

#### Creation science trial

#### Verdict awaited

Washington

It may be another week before the verdict is known on the creationist trial which ended in Little Rock, Arkansas, last Thursday. Initially, Judge William Overton had promised an immediate verdict on whether a new state law requiring equal time for the teaching of evolution and "creation science" in state schools was unconstitutional.

At the end of the two-week trial, however, the judge announced that the amount of evidence presented was so large that his verdict would be delayed, although he has promised to deliver it by 31 December.

Despite the delay, the American Civil Liberties Union (ACLU), which brought the case on behalf of several local religious groups, biology teachers and school children, is confident that it has won. "It was no contest," Mr Bruce Ennis, the chief ACLU attorney, said after the trial had ended. "The state did what it could do. It was inadequate not because it did not do its job, but because creation science is a religion."

Supporters of creation science also

seemed to be accepting their defeat. But in this case the blame was being placed on the performance of state attorney general Steve Clark in defending the creation science law. The creationists promise a tougher fight in the next court case, which is likely to be a similar challenge against a creation science law passed in the state of Louisiana.

Although Judge Overton has yet to declare his verdict, he did say that it would be limited to the question of whether the creation science version of the origins of the world was religion, despite any explicit religious or biblical references in its description in the Arkansas law.

He added that he would not undertake to decide the validity of the biblical version of creation nor the theory of evolution. ACLU has asked the judge to determine various "findings of fact" — such as the definition of a scientific theory being based on natural laws and being "explanatory, testable and tentative" — which it hopes can be used in future court battles.

The second week of the trial was taken up largely by various witnesses called by the state to present a case in favour of creation science and the Arkansas bill, virtually identical copies of which are now pending before almost 20 other state legislatures.

Cross-examination by ACLU attorneys provided some colourful testimony. One supporter of creation science, having described how a creator could still be a scientific concept, perhaps comparable to Aristotle's "first cause", went on to describe his belief in exorcism and unidentified flying objects, claiming the latter to be attacks by Satan on God's world.

The star witness for the defence was Professor N.C. Wickramasinghe, head of the department of mathematics and astronomy at the University of Wales in Cardiff. Professor Wickramasinghe told the court that the odds against life originating by chance anywhere in the Universe were so high as to be virtually impossible. "One is driven almost inescapably to accept the possibility that life results from deliberate creation", he said.

He claimed that his own theories about the possible existence of microorganisms on comets bringing life to Earth had been rejected by other scientists largely because of their "indoctrination in Darwinism".

But if such statements were music to the ears of the creationists, there was less consolation when Professor Wickramasinghe was asked to comment on the creation science law, when he claimed that most of it was "claptrap", and that "certain parts of the law are demonstrably wrong".

One of the scientific witnesses who had been expected to appear for the defence unexpectedly left town shortly before he was due to testify. Another scientific witness whose appearance was cancelled by the state was Henry D. Voss, an electrical engineer who has published papers on space physics.

David Dickson

# CORRESPONDENCE

# Voices from a wilderness

Sir — We appeal for help to the editorial staff of *Nature*, to our colleague scientists and to the World Federation of Scientific Workers.

We are research workers of Jewish origin. More than two years ago, we came to the decision to leave the Soviet Union and accordingly applied for permission for our families to leave the country for Israel. Soviet law and present practices gave us reason to hope for a quick departure from the country since none of us has ever dealt with state or military secrets or with any questions connected with the security of the Soviet Union. Yet for more than two years we have been denied permission to leave, without reference to the Soviet legal code and without legal or lawful justification.

It seems that considerations in big politics do not take human lives into account.

We doubt whether our colleagues in the West realize what happens if a scientist in the Soviet Union declares his decision to leave the country. Almost all of us have lost our jobs and we are deprived of any possibility of continuing with our scientific research. The doors of scientific institutions, laboratories, seminars, conferences and symposia, editorial offices and publishing houses are shut in our faces. We are being deprived of our scientific degrees.

We feel that the danger of our creative death is becoming more and more real. Any scientist will understand what a two or three year break in scientific work means; and nobody can tell us how long our deprivation will last. Nobody is interested in our qualifications; nobody needs our work here.

Having lost our jobs, we find ourselves in a hard and humiliating financial position. We are anxious about the fate of our families; many of us have small children. We have in fact been thrown out of society, but at the same time we are not allowed to leave the Soviet Union.

All this has naturally had a harmful effect on our health — nervous depressions, hypertonic crises and other diseases have become our permanent companions. Our children are also affected.

We are in a situation where we can count only on the support and solidarity of our colleagues abroad, on their help and sympathy. Our voice in the Soviet Union is really a voice in the wilderness, our fate is of no interest to anybody and nobody answers

our complaints or petitions.

We understand that you all face important problems of your own, but hope that the fate of individual scientists — your colleagues — is also important and not a matter of indifference to you. For the world of science is common for everyone; it has common ethics and morals.

Indeed, our fate is closely connected with the attainment of lawfulness, the observance of the norms and rules of international pacts and human rights declarations, with the fulfilment of the Helsinki agreements and with adherence to humanitarian principles — with everything that makes possible mutual trust and mutual understanding between nations.

We are sure that struggle for the attainment of these principles is part and parcel of the struggle for peace and detente.

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**SERC's plans** 

Sir — In your excellent leading article of 26 November (p.295) you state that the Science and Engineering Research Council "has just put off a plan to break new ground in the fashionable field of molecular electronics". It is certainly true that we are unable to fund in an adequate fashion programmes that certainly deserve support, but in the particular case of molecular electronics we are currently planning to increase our support of research in this potentially exciting area. Inevitably this means we have to cut back on other deserving areas.

B.W. OAKLEY

Science and Engineering Research Council, Swindon, UK

#### An acid test

Sir. — The objection of Darnbrough et al. (Nature 26 November, p.294) to data in our BioSystems paper on the origin of life is based, according to correspondence, on experiments with ATP at pH 11, where ATP is unstable. On page 162 of the article, which they cite inaccurately, experiments at pH 7.2 are described. This technical detail (that is, experiments at pH 7.2) is critical in judging their statements about plausibility. For readers interested in confirming this, the correct reference is Fox and Nakashima, BioSystems 12, 155–166; 1980.

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# Literary anarchy

Sir.— The problems raised in recent letters (7 May, p.7, 28 May, p.278) about literature search and by Dana L. Roth (8 October, p.422) about recruiting librarians with subject expertise may be solved if and when today's librarians turn towards Anarchism as in the following: "How to be an Anarchistic but Indispensable Librarian".—

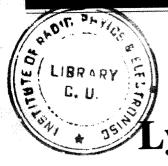
- Make your own rules so that you can change and interpret them according to circumstances.
- (2) Always act contrary to the rules
- (3) Use flexible opening hours; go out as often as possible so that your readers are happy if and when they see you.
- (4) Differentiate your catalogue cards.
- (5) a. Articles in authors' names may or may not be written after the names; sometimes split names are typed as whole ones.
  - b. Use alternatingly full and abbreviated journal titles; use different abbreviations for the same title.
  - c. Keywords are typed above the author's name or underlined; singular, plural, adjective and complex word forms are to be used.
  - d. Never use see- or see also-references.e. Never correct errors.
- (6) Foreign characters are alternatingly transliterated or not.
- Greek alphabet: transliterate or write in full (y = g or gamma).
- (6) 2. Figures are alphabeticized before or after plain characters but they may also be written in full in different languages.
- (6) III. Chemical substances will be found alternatingly under the common name, the scientific name, the product formula or the gross formula.
- (7) Print a catalogue of current journal titles but do not include the years or volume numbers your collections start with.
- (9) Move your books and journals regularly.
- (X) Take holidays without warning anybody; never tell the date of your return.
- (11) Never compose statistics with exact numbers but make rough estimates.
- (12) Avoid management techniques (whatever they may be).
- (13) Help your readers as well as possible; talk at least half an hour with every new customer and at least one hour a week with regular visitors.
- (14) Be fraternal: send now and then somebody on to other libraries
- (eight) Never write anything down but don't try to remember everything either.
- (Four) Make sufficient catalogues:
  monographs and reprints each have
  separated authors, journals and serials,
  systematic, and keywords catalogues;
  put all these catalogues in one piece of
  furniture.

If you do this and a lot of other things, you deserve the title "Anarchistic Librarian"; but: your customers will be satisfied and you have made yourself indispensable.

G. MERTENS (Anarchist) Librarian

Katholieke Universiteit, Leuven, Belgium

# NEWS AND VIEWS



# Lymphokines on the move

from Verner Paetkau

LEUKOCYTES which are stimulated with mitogens or strong antigens have long been known to secrete mediators with profound effects on other leukocytes. Non-immunoglobulin protein products with effector properties in the immune system have been designated 'lymphokines' and have a wide spectrum of activities. It is perhaps not surprising that, until recently, only a few immunologists were interested in these mixtures of soluble mediators — they were apparently antigen-nonspecific and poorly defined. How things can change! Today, the lymphokines and related soluble mediators command the serious attention of both theoretical and practical immunologists. Several lymphokines and their relevant cells have been characterized, and a picture is emerging of discrete molecular entities acting in defined (or definable) ways. In addition, the factor identified as interleukin-2 (IL2), which stimulates proliferation of T lymphocytes, is used to generate clonal sources of T lymphocytes with specific cytotoxic or helper activities. Today, the interleukins are a growth industry.

The present phase of work began in the mid 1970s and results from the convergence of several lines of enquiry. The sources of factors were normal leukocytes stimulated with mitogenic plant lectins such as concanavalin A (Con A) or phytohaemagglutinin. Not unexpectedly, the conditioned culture media used in early experiments contained a variety of activities, and different laboratories concentrated on different lymphokine assays. In one case, a soluble mediator greatly enhanced normal mitogenic responses of thymic lymphocytes to certain plant lectins. In another, lymphokine preparations generated from mitogenstimulated leukocytes were found to induce the continuous proliferation of functional T lymphocytes<sup>2,3</sup>. Similar preparations could also replace T lymphocytes in antibody-forming responses of B lymphocytes4, an activity referred to as 'T cell replacing factor' (TRF).

By 1978, it was evident that at least two molecular entities could be defined. One, a product of macrophages or monocytes, had been termed 'lymphocyte activating

factor'. The factor responsible for this activity has been renamed interleukin-1, and was recently purified to homogeneity<sup>5</sup>. The other, with effects on thymic lymphocytes, cytotoxic lymphocytes and T-depleted B lymphocytes, bore a number of designations, depending on the assay and laboratory. A single factor appeared to be responsible for this group of activities, there being no resolution by gel filtration, ion exchange chromatography, or isoelectric focusing. It was designated interleukin-2 (for T cell growth factor, thymocyte stimulating factor and costimulator). This was more than an exercise in semantics; it helped to focus the work and to give it some testable tenets. Because both biochemical and biological criteria for distinguishing IL2 and IL1 existed, it was possible to look for other activities in crude culture supernatants. Recent progress has been both stimulating and satisfying.

A soluble mediator which induces an enzyme activity (20-a corticosteroid dehydrogenase) associated with mature helper T lymphocytes has now been characterized by Hapel and co-workers6, and designated IL3. Conveniently, IL3 and IL2, which are both present in Con A-generated supernatants of normal spleen cell cultures, are separable by ion exchange chromatography. IL3, which is demonstrably free of IL2, interferon and TRF, stimulates the clonal outgrowth of T lymphocytes with surface antigens expected of T helper cells. Indeed, the clones eventually begin to generate their own IL3 and become able to grow independently of added factor. These putative T helper cells produce IL2 when stimulated with phorbol myristate acetate (PMA), an agent previously shown to elicit IL2 production from normal splenic T (helper) lymphocytes7. PMA apparently overrides the normal requirement for IL1 and antigen/mitogen for IL2 production8. Given the availability of biochemical separation methods and clonal cell lines for assay, one can soon expect a further

understanding of T cell signal networks, in which the roles of interferon, colony stimulating factor, IL1, I-A recognition, IL2, IL3, TRF and antigen will be identified more precisely.

The question of whether IL2 acts directly on B cells when it restores T cell function in antibody responses has not been answered definitively. The difficulties can be illustrated by recent work in which spleen cells of athymic mice were stimulated to grow by IL2 preparations, and in the process generated T lymphocytes (Lipsick and Kaplan, ref.9). This observation is consistent with previous results showing a recovery of T cell function in vitro and in vivo with IL2. The recovered T cell function may in turn be responsible for the restoration of T-dependent B cell function.

Does this report show, in contrast to what had been thought, that IL2 is directly mitogenic for T cells or their precursors in the absence of a second signal (antigen or mitogen)? The difference from earlier reports may be less significant than it seems. It is known, for example, that IL2 has no direct mitogenic effect at low cell density. In the Lipsick and Kaplan work. spleen cells were cultured at high density (5 million per ml) in serum-free medium; serum both enhanced background mitogenic activity and reduced that stimulated by IL2. A significant question remains — is the activity in fact due to IL2? Or, in light of the results of Hapel et al. 6, is IL3 at work? Although most of the experiments were performed with relatively crude IL2, which may well contain IL3, the same results were apparently obtained with preparations which should be free of IL3. Further direct evidence on this point will be welcome.

The question of direct effects of IL2 on B cells may be partly resolved by the finding that IL2 preparations contain a discrete factor which stimulates B cell proliferation<sup>10</sup>. Similar results are forthcoming from laboratories at the NIH (Bethesda) (M. Howard et al., personal communication). The B cell growth factor has been separated from IL2 (ref. 11). In the model studied, the NIH workers feel that IL2 is synergistic with B cell growth factor.

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Finally, what of TRF4? After many years of difficulty, created in part by the ability of factors like IL2 and the B cell growth factor to replace T cells indirectly, assays which clearly distinguish between IL2 and TRF are now in hand. And the take-home message is that, indeed, TRF exists<sup>12</sup>. It acts at the time when B cells, having undergone clonal expansion following antigenic stimulation, are to begin secreting high levels of immunoglobulin. Then, as proposed years ago by Dutton and colleagues, TRF can replace T cells in switching on a high level of immunoglobulin synthesis. Using spleen cells thoroughly depleted of T cells (this requires treatment of the donor animal with anti-thymocyte serum, in addition to the more common treatment with anti-Thy-1 antibody in vitro), Swain and coworkers have demonstrated synergy between IL2 and TRF in generating plaque-forming B cells. The role of IL2, although not yet delineated in this system, may be related to its ability to induce T cell

functions early (proliferative phase). The relationship to the newly described B cell growth factor awaits further experiments. In both cases, a clearer understanding of the roles of several lymphokines now exists, and perhaps more important, the tools and approaches necessary for more sophisticated analysis are evident.

An indication of future directions is

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given by a current report describing a start on molecular biological studies of IL2. Given several T cell tumour lines which can be induced to generate high levels of IL2 (several thousand times the amounts produced by normal lymphocytes), it has been possible to follow the lead of workers in the interferon field, by looking for mRNA coding for the lymphokine, and then attempting to clone its complementary DNA. The EL4 variant cell line originally described by Farrar and colleagues at NIH<sup>13</sup> does indeed contain demonstrable IL2 mRNA. The poly  $(A)^+$ , 11-12S RNA derived from PMA-stimulated cells of this line, injected into Xenopus laevis oocytes, directs the synthesis of biologically active IL214. Similar results have been obtained by Gillis and colleagues in Seattle. The next phase of work clearly will involve molecular biological approaches. With venture capital panting for release, one may also expect that the term 'growth industry' could soon apply to lymphokinology in more than one way.

# A new view of supernova remnants in the Magellanic Clouds

from David Clark

FEW areas of astrophysics have not seen major changes following the highly successful Einstein X-ray observatory mission. The impact on the study of supernova remnants (SNRs) has been particularly impressive. When a massive star dies as a supernova, a shock wave is driven outwards from the site of the explosion, sweeping-up and heating interstellar gas to the millions of degrees required for thermal X-ray emission — so forming an extended X-ray remnant with characteristic peripheral brightening. Gas cooling behind the shock may be seen to radiate optically, and SNRs may also be detected at radio wavelengths. The radio emission is nonthermal, produced by fast electrons trapped in the interstellar magnetic field compressed by the expanding shock wave.

Because X-ray astronomy is a comparatively young science, pioneering surveys of SNRs in our own Milky Way, and its closest galactic neighbours, the Magellanic Clouds, were carried out in the radio and optical. Within the Galaxy more than 120 extended non-thermal radio objects were identified as SNRs. Detectable optical nebulosity was found only in the 30 closest of these because of the obscuring effect of dust permeating the plane of the Galaxy. A common characteristic of the optical remnants was recognized — the intensity of the red line emission associated with singly ionized sulphur with respect to the intensity of the red hydrogen Balmer line was very much greater in the shock-ionized plasma of SNRs than in photo-ionized nebulae. The combination of non-thermal radio emission and strong sulphur emission was taken to be a unique signature of SNRs,

and was used to detect remnants in the Magellanic Clouds and other nearby galaxies.

The pioneering survey of SNRs in the Magellanic Clouds by Mathewson and Clarke in Australia in 1973 used the radio/optical technique described above. The survey identified 14 remnants in the Large Magellanic Cloud (LMC) and 3 in the Small Magellanic Cloud (SMC), and was assumed to be almost complete (although a few other ring-like nebulosities seen in a deep optical survey of the Clouds by Davies and co-workers in 1976 were proposed as possible remnants). That assumption has been shattered by the X-ray survey of the LMC completed by Einstein and recently reported by Long, Helfand and Grabelsky (Astrophys J. 248, 925; 1981). Einstein detected X-ray emission from 13 of the known 14 LMC remnants, plus 12 other objects which are certain SNRs (5 of which were also in the Davies et al. list), plus 25 further objects which are possible SNRs on the basis of existing X-ray data.

The implications of these new discoveries are very significant. They seriously question the sole use in extragalactic SNR surveys of the traditional radio/optical SNR detection technique as it revealed perhaps just one-quarter of the LMC population of remnants. Optical nebulosity associated with many of the new remnants showed sometimes no sulphur emission - sometimes no hydrogen

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emission. The new detections allow the supernova rate in the LMC to be lowered to perhaps one per century, contrasting with earlier estimates of one per 400-900 years. But most significant, the major population of LMC SNRs now identified in X rays and optically are found to be radio 'subluminous'; this result must call into question many of the studies of remnant evolution, distribution, ages, birth rates, distances and so on in our own Galaxy since such studies are based almost entirely on radio data. The new uniform LMC sample of SNRs should prove invaluble in future studies of remnant evolution at all wavelengths.

Observations of the six brightest LMC remnants using the solid-state spectrometer on Einstein are to be published shortly. These too show unexpected results. Four of the six display characteristic thermal spectra expected for shock-heated interstellar material; but the other two show non-thermal spectra suggestive of continued energy input. This is the case for the well known SNR the Crab Nebula, which in addition to emitting non-thermal X rays is brightest at its centre rather than peripherally, the emission being driven by an active pulsar. One of the LMC remnants, going by the rather unspectacular name of N157B, also shows unusual central brightening (as Australian radioastronomers showed several years ago) and mimics the Crab Nebula in other ways, including the non-thermal X-ray emission. It is the first Crab-like remnant detected in another galaxy, and must represent a prime site for the detection of the first extragalactic pulsar.

#### Are stress fibres contractile?

from Keith Burridge

Interest in stress fibres arises in part because their organization resembles the structure of striated muscle myofibrils. Not only do they contain many of the same proteins (for example, actin, myosin,  $\alpha$ -actinin and tropomyosin) but many of these are arranged in an alternating periodic pattern similar to that seen in sarcomeres. The similarity to muscle structure has suggested that they may have a similar function and be involved in non-muscle cell movement.

Here, however, is a paradox. Stress fibres are not required for cell movement or migration, and rapidly moving cells such as macrophages or amoebae lack them. Couchman and Rees1 have shown that fibroblasts migrating from primary tissue explants generally lack stress fibres in the first few days when they are migrating rapidly. With time the migration of the cells slows down and stress fibres become more prominent. The correlation of lack of movement with the presence of stress fibres has been best documented by Herman, Crisona and Pollard2. Fibroblasts migrating randomly in culture were filmed, then fixed and prepared for immunofluorescence with antibodies against actin, and examined for the presence or absence of stress fibres. Cells or regions of cells moving most actively appeared to contain no stress fibres while regions of cells with prominent stress fibres moved very little.

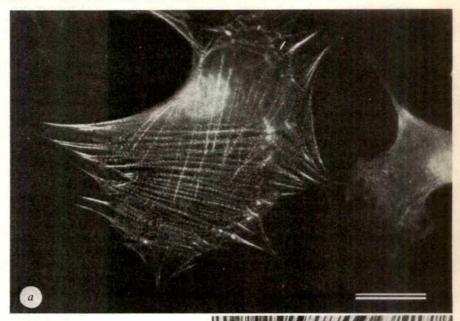
Because of the similarity of stress fibres to myofibrils, several groups have looked for contractility. Isenberg and co-workers3 demonstrated that stress fibres which have been microdissected from living cells with lasers will contract in the presence of Mg2+-ATP. An elegant approach was used by Kreis and Birchmeier4 who microinjected fluorescently labelled α-actinin into living cells so that it would be incorporated into the stress fibres. Contraction was then induced in permeabilized cell models with Mg2+-ATP. During such contractions, shortening of the non-fluorescent space between the fluorescent regions of a-actinin was observed, consistent with a sliding filament mechanism like that found in muscle. Contraction was not, however, observed in live cells as they first had to be permeabilized. Clearly these structures are potentially contractile, but what is their function in intact cells?

It would be helpful to know under what physiological circumstances stress fibres are found in the body but, unfortunately, rather little information is available. Stress fibres have been described in aortic endothelial cells of hypertensive animals5 and also in the granulation tissue of wounds6 but fibroblasts in many normal tissues do not contain prominent stress fibres. Growth of fibroblasts in culture seems to promote the development of stress fibres, perhaps reflecting unphysiological conditions of the culture environment. Unlike conditions in vivo, cultured cells grow on a rigid substrate to which normal fibroblasts adhere strongly. Some years ago, Willingham and his coworkers7 showed that when the substrate was weakly adhesive for the cells, they did not spread and did not form stress fibres. When the same cells were induced to adhere tightly, they flattened on the substrate and prominent stress fibres appeared. Tight adhesion to the underlying substrate appeared to be the key to their appearance. Couchman and Rees1 reached a similar conclusion in their study of fibroblasts migrating from explants.

How might tight adhesion to a substrate induce the formation of stress fibres? One possibility is that the tight adhesion itself restricts or sterically inhibits the local movements of surface membranes. In turn, this might permit an ordering of the adjacent cytoskeletal elements into stress fibres — one that would not occur in a

region of cytoplasm that was in a more dynamic state, such as that underlying a ruffling membrane. An alternative explanation I find preferable is that stress fibres arise within a cell because it attempts to pull against a point of tight adhesion to the substrate. Since the substrate is a plastic culture dish, tension will develop. This will result in the alignment of individual microfilaments and possibly also in the recruitment of additional filaments to the bundle that develops. Thus the more strongly a cell adheres to a substrate the greater is the tension and the larger the resulting stress fibre. Several lines of evidence are consistent with this view.

Wohlfarth-Botterman and Fleischer8,9 showed that tension will induce large bundles of microfilaments in the protoplasmic strands of Physarum when these are made to contract under isometric conditions. The microfilament bundles, reminiscent of the stress fibres of fibroblasts, are not induced, however, during isotonic conditions. Physarum is phylogenetically far from cultured fibroblasts, but it may reveal a principle of non-muscle actomyosin systems. When stress fibre contractility in fibroblasts has been studied, isotonic contraction (that is, shortening) has been looked for, but the failure to observe this in live cells is hardly surprising if tight adhesions to the substrate make shortening impossible. Strong adhesions would impose isometric conditions on a contraction if the



Stress fibres in cultured cells viewed by immunofluorescence microscopy. a Shows a gerbil fibroma cell stained with an antibody against a-actinin. b Shows part of a large human fibroblast stained with an antibody against tropomyosin. Bar in a represents 20  $\mu$ m; b is at one-half this magnification.

Keith Burridge is an Assistant Professor in the Laboratories for Cell Biology, Department of Anatomy, 111 Swing Building 217H, University of North Carolina at Chapel Hill, North Carolina 27514. adhesions do not break. Only when these adhesions have been weakened, as might be expected in cell model studies3,4, would shortening of stress fibres be observed.

That tension on the substratum is generated has been shown by Harris and co-workers10,11, who introduced flexible silicone rubber substrates to study cell movement. Highly motile cells, such as leukocytes, are unable to deform the flexible substrate during their movements. but more sedentary cells such as fibroblasts cause extensive wrinkling of the rubber, indicative of considerable tension. It seems most likely that stress fibres are the cellular structures responsible for generating this tension. Cells without stress fibres, even though they may move rapidly on the flexible substrate, appear unable to generate sufficient tension to wrinkle it. On the other hand, those cells that induce wrinkles beneath them tend to show large microfilament bundles often running at right angles to the wrinkles (unpublished observations). If the cells are rounded up with trypsin, the wrinkling (that is, the tension) is released11 and others have shown that these conditions also result in the rapid disassembly of stress fibres (see, for example, ref. 4). To generate the wrinkle some shortening of the stress fibres must occur; but even here a considerable element of the contraction must be isometric, since once formed the wrinkles are held for long periods of time, although they will snap back if the cell's adhesion to the rubber is released, indicating a sustained tension both in the rubber and the cellular elements causing it to wrinkle.

In conclusion, it would seem that stress fibres have little to do with cell migration. Several lines of evidence suggest that these structures are contractile but that in cultured cells this contraction is isometric rather than isotonic. It seems likely that the tension generated at points of tight adhesion to the substrate contributes to the formation of these bundles of actin microfilaments. In future it will be important to investigate further the conditions in which stress fibres appear in the body, and it will be particularly relevant to examine locations where tight adhesion and isometric tension might be expected to occur.

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# Order or disorder in the structure of glass?

from S.R. Elliott

THE question posed in the title has provided a recurrent theme in the study of the structure of non-crystalline solids since the seminal work of Zächariasen in 1932. The absence of long-range order (periodicity) is a prerequisite for an amorphous material. The point at issue. therefore, is what degree of short- or medium-range order is present, if at all, in a glass? Is the structure of non-crystalline materials completely random and chaotic, or are there, as Zachariasen supposed for covalent materials, well defined structural units (for example, SiO<sub>4</sub> tetrahedra in SiO<sub>5</sub> glass) which are themselves randomly connected together?

Much evidence from diffraction and other experiments shows that covalently bonded amorphous solids do possess a strong degree of local order (that is, structural units), but controversy still exists over the degree of medium-range order whether the units are completely randomly connected together, or whether there exist preferred orientations, just as in the crystalline forms.

It is generally accepted, following Zachariasen, that the structure of directionally bonded (that is, covalent) amorphous solids can be well represented by a 'continous random network' (CRN), in which say for SiO<sub>2</sub>, SiO<sub>4</sub> tetrahedra are connected together at the apices through the oxygen atoms, such that every Si is surrounded by four O atoms, and every O is surrounded by two Si atoms, although the bond-angle subtended at the O atom may subtend a wide range of values. The SiO<sub>4</sub> tetrahedra is relatively well defined but there exists some uncertainty in the Si bond-angles, and the relative orientation of units is not at all well defined. A further complication arises for multicomponent systems since a single diffraction experiment only gives an average picture of the structure, being a sum of say, the individual Si-Si, Si-O, O-O pair correlation functions, so that only a limited amount of unambiguous structural information can be obtained.

For these reasons, a recent paper by Daniel and Leadbetter1 makes an interesting contribution. They studied an amorphous elemental system, arsenic, by means of X-ray diffraction. Amorphous arsenic (a-As) has been much studied in the past (see ref. 2 for a review), and is of interest because of its position in the Periodic Table midway between a-Si (used for solar cells) and a-Se (the active component in the Xerox process). However, in contrast to previous studies, Daniel and Leadbetter studied the molecular form of a-As. It is well known that As vapour consists of tetrahedral As<sub>4</sub> molecules, and if this vapour is condensed on a substrate held at 30K, an amorphous

form is produced consisting of a packing of As, molecules, bonded together by van der Waals' forces. Thus, in this case, the local order is extremely well defined (that is, the As<sub>4</sub> tetrahedron), and in principle the medium-range order of the amorphous solid can be explored directly, since the local structure is known accurately. A model whereby all the As4 tetrahedra are randomly oriented does not give peaks in the angular dependence of the scattering intensity in the same positions as those observed experimentally, implying there is a considerable degree of correlation between molecules in the way they pack. Instead, it appears that the molecules of As<sub>4</sub> prefer to pack in a slightly distorted, staggered face-to-face configuration. Similar substantial correlations are found in other (liquid) molecular systems, such as P and CC1<sub>4</sub>.

Another interesting point is the observation that molecular amorphous arsenic is structurally unstable, even at temperatures as low as 77K. The highly strained As<sub>a</sub> molecules, with bond-angles of 60°, irreversibly polymerize to form a cross-linked network, which has an X-ray diffraction pattern very similar to that of bulk a-As, which has a bond-angle of 98° and whose structure can be well simulated by a CRN<sup>2</sup>. This amorphous-amorphous transition is interesting in that it involves a distinct change in medium-range order: peaks in the real-space correlation function separate much further in the molecular form than in the polymerized material.

This work has considerable bearing on another commonly studied amorphous system, the As, S, system. This, unlike As itself, readily forms a glass by quenching of the melt, but can also be formed into amorphous thin films by vacuum evaporation. However, the stable vapour species is the almost spherical As<sub>4</sub>S<sub>4</sub> molecule, not the As<sub>4</sub>S<sub>6</sub> molecule which would preserve stoichiometry, and indeed Daniel and Leadbetter in an earlier paper<sup>3</sup> studied the structure of evaporated amorphous thin films of 'As,S,' and concluded that the structure is composed of a packing of As<sub>4</sub>S<sub>4</sub> molecules. Just as in the case of elemental As films, the As<sub>4</sub>S<sub>4</sub> molecules appear to pack in a highly correlated manner, but detailed interpretation is made difficult because of the two atomic species present and also because of the spherical nature of the molecule concerned.

Evaporated thin films of As<sub>2</sub>S<sub>3</sub> (or other alloys containing S or Se) are interesting because they exhibit both thermally and photo-induced structural changes. Like molecular As, the As-deposited films polymerize irreversibly on annealing, the

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material becoming more chemically ordered in the process, as shown by X-ray diffraction<sup>3</sup> and EXAFS studies<sup>4</sup>. These materials are semiconductors, and irradiation by band-gap light also serves to induce polymerization, the so-called 'irreversible photostructural effect'. More mysterious, however, are the reversible structural changes induced by band-gap radiation on well annealed films and melt-quenched glasses of the same materials. These structural changes, on the scale of about 6Aappear to be smaller than those encountered in the irreversible effect and are accompanied by various optical

changes<sup>5</sup>; they can be annealed away by heating the sample to the glass transition (softening) temperature, whereupon reexcitation induces the same effect. The nature of these structural changes remains completely unknown. Perhaps when we understand the medium-range order present in glasses we might begin to understand this effect too!

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# Calcium and calmodulin in Kyoto

from Robert S. Adelstein

Many diverse biological processes are regulated by the concentration of intracellular calcium, including cell motility and the synthesis and degradation of biologically important peptides and nucleotides. The effects of calcium are often mediated by the small (molecular weight 16,500), acidic protein calmodulin, which appears to be ubiquitous throughout much of the plant and animal kingdoms. Calmodulin contains four binding sites for calcium which become occupied at a calcium concentration of 10-6 M, a level corresponding with the onset of many calcium-mediated processes in cells. In marked contrast to the calcium-free molecules, the complex of calcium and calmodulin is capable of binding to and actvating numerous proteins with different biological activities. The mechanism and consequences of the binding of calcium to calmodulin were discussed in a meeting\* in Kyoto, Japan this past summer.

There was general agreement that positive cooperativity has a role in binding the second Ca<sup>2+</sup> to calmodulin (K. Yagi, Hokkaido University; J. Haiech, Montpellier University). It was further proposed that the binding of all four Ca<sup>2+</sup> to calmodulin is not random, but occurs in a specific sequence. Two enzymes that are activated by calmodulin, cyclic nucleotide phosphodiesterase and myosin kinase, appear to require the species with four Ca<sup>2+</sup> bound.

A major theme recurring throughout the meeting was the interaction between two major intracellular regulators: Ca<sup>2+</sup>, which is often coupled to calmodulin, and cyclic nucleotides, which are coupled to enzymes catalysing covalent phosphorylation. The kinetic analysis of the regulation of cyclic nucleotide phosphodiesterase by calcium and calmodulin was

presented by J. Wang (University of Manitoba) and his model used to explain the highly cooperative activation of phosphodiesterase by Ca<sup>24</sup> -calmodulin.

In the control of serotonin biosynthesis (Yamauchi and Fujisawa, Asahikawa Medical College), both tyrosine and tryptophan hydroxylases appear to be regulated by a Ca2+-calmodulindependent phosphorylation which promotes the binding of a separate activator protein. This heat-labile activator has a molecular weight of 70,000, is composed of two subunits of 35,000 and binds to the phosphorylated species of hydroxylase only, bringing about a twofold increase in enzyme activity. Additional activation by cyclic AMPdependent phosphorylation at other sites provides a separate mode for regulating these enzymes.

Y. Takai and Y. Nishizuka (Kobe University School of Medicine) described a calcium-dependent but calmodulin-independent membrane-bound kinase. This enzyme, which has now been purified from rat brain to near homogeneity, has a molecular weight of 77,000 and has been named C-kinase. It requires micromolar concentrations of Ca2+ and phospholipid for its activation, but contains no calmodulin, nor is it activated by exogenous calmodulin. Instead, a small quantity of unsaturated diglyceride increases the affinity of enzyme for Ca2+ as well as for phospholipid. The hydrolysis of phosphatidylinositol to phosphatidylserine and diacylglycerol, which can be induced by a number of different extracellular messengers, is directly coupled to its activation. The presence of relatively large amounts of protein kinase C activity in brain, platelets and other tissues and the interaction of this system with the cyclic GMP-regulatory system, suggests great promise for further studies.

Ca<sup>2+</sup> - and cyclic nucleotide-mediated reactions appear directly coupled at the level of their receptor proteins (C. Klee,

NIH). In brain extracts, calmodulin interacts in a Ca<sup>2+</sup>- dependent fashion with the regulatory subunit of cyclic AMP-dependent protein kinase.

One of the best examples of the coupling of the Ca<sup>2+</sup>-calmodulin regulatory systems with cyclic AMP-mediated regulation is that of smooth muscle. Myosin kinase, the enzyme regulating phosphorylation of smooth muscle myosin, is a substrate for cyclic AMP-dependent protein kinase (R. Adelstein, NIH) and phosphorylation of myosin kinase decreases its activity by decreasing its ability to bind calmodulin.

The role of calmodulin in regulating muscle contractile proteins was discussed by several investigators. Calmodulindependent phosphorylation of the 20,000 molecular weight light chain present on both heads of a single smooth muscle myosin molecule appears to be required before the Mg2+-ATPase activity of either head can be activated by actin (D. Hartshorne, University of Arizona). Using tracheal smooth muscle (J. Stull, University of Texas) an increase in tension was found to be paralleled by an increase in myosin phosphorylation; however, consistent with the results of Dillon et al. (Science 211, 495; 1981), it was possible to maintain isometric tension despite a decrease in the state of myosin phosphorylation.

S. Kakiuchi (Osaka University Medical School) presented evidence that calmodulin binds to spectrin, a component of the erythrocyte membrane, and suggested that the interaction might be involved in maintaining the normal shape of the red cells. He also reported the presence in gizzand smooth muscle of a novel protein (molecular weight 150,000) that binds to calmodulin in the presence of  $10^{-6}$ M Ca<sup>2+</sup> but binds to actin at low (< $10^{-7}$ M) Ca<sup>2+</sup> concentrations. The protein might inhibit the ability of actin to polymerize at low Ca<sup>2+</sup> concentrations.

Pharmacological aspects of the calmodulin system opened the meeting. F. Vincenzi (University of Washington) reappraised the action of neuroleptic drugs, particularly phenothiazines on calmodulin function. Some presumed calmodulin antagonists as well as neutral and acidic lipids seem to act directly on activatable enzymes, possibly interacting with hydrophilic regions of calmodulin-binding sites.

H. Hidaka (Mie University Medical School) presented studies of naphthalene-sulphonamide derivatives (W-7 and W-5) as antagonists of calmodulin action. These ampiphilic compounds appear to be more selective for calmodulin than phenothiazines with much less effect as general membrane perturbants. Treatment of cultured Chinese hamster overy cells

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The symposium on Revent Advances in  $Ca^{2+}$  and Cell Function; Calmodulin and Intracellular  $Ca^{2+}$ -Receptors was held in Kyoto, Japan and organized by S. Kakiuchi (Osaka University Medical School) and H. Hidaka (Mie University School of Medicine).

with W-7 at moderate concentrations was shown to prohibit cell growth, arresting the cells in late G<sub>1</sub> phase. Using <sup>3</sup>H-labelled W-7, its distribution *in vivo* was shown to be similar to that of calmodulin under

conditions where growth arrest was obtained — suggesting that calmodulin may provide Ca<sup>2+</sup> -dependent regulation of functions required for the cells commitment to replication and division.

carcinogenicity is, in general, independent of metabolic action.

# Environmental nitrosamines and cancer

from Valda M. Craddock

It is now twenty-five years since dimethylnitrosamine was shown to cause liver cancer in rats in the historic experiments of Barnes and Magee. Tumours have now been shown to be induced by a wide variety of N-nitroso compounds in many species of animal and there is no reason to believe that man will prove to be an exception. There is also no doubt that man is exposed to these potent carcinogens. They frequently occur in industry and are widespread in the environment. Clearly, it is important to find out where the nitroso compounds come from, whether the levels found represent a hazard for man, what can be done about them and what the cellular and molecular mechanisms are by which they cause cancer. Progress in these areas was

discussed at a recent conference\* in Japan.

At previous meetings, emphasis had been on methods for the detection and estimation of nitrosamines when present in complex biological material. In the past, several reports of nitrosamines in the environment have proved to be false alarms when more specific analysis showed the presence of related chemicals. The situation at present is that volatile nitrosamines can be analysed by gas chromatography/thermal energy analysis. With non-volatile nitrosamines, preliminary formation of volatile derivatives is necessary.

N-nitrosoamines are likely to be formed wherever amines, secondary or tertiary, encounter nitrite. N-nitrosamides result from similar reactions with amides. Both types of compound are potent carcinogens. Nitrosamines are relatively stable and require metabolic activation to form the ultimate carcinogen; nitrosamides on the other hand are unstable, and the site of

Much good work done in detecting environmental nitrosamines comes from the German Cancer Centre (Preussmann, Heidelberg). Nitrosamines in beer were first detected in Germany. They are formed by the reaction of amines in barley with nitrous fumes from the fuel used to heat barley during the malting process, and can be dramatically reduced by changing the source of the heat. The nature of the amines involved is being elucidated (Wainwright, Brewing Research Foundation, Nutfield). Nitrosamines have been detected in rubber, including the rubber used in the manufacture of babies' dummies (Spiegelhalder and Preussmann, German Cancer Centre, Heidelberg). The use of alternative retarders, or of 'safe' amines, should reduce this hazard. Relatively high concentrations of nitrosamines have been found also in cutting oils (Keefer, NIH), and it is again possible to suggest methods for their elimination. Cosmetics are known to contain nitrosamines, and here the situation is less clear. The fact that female facial skin is not a high cancer incidence area is not necessarily a reassurance; N-nitroso compounds can be absorbed through the skin and cause cancer elsewhere. On the whole, however, the meeting was

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\*\*Seventh International Meeting on N-Nitroso Compounds: Occurrence and Biological Effects\*, held in Tokyo, 28 September-1 October 1981, under the sponsorship of the International Agency for Research on Cancer, France, and the Japanese Cancer Association, Tokyo.



#### 100 years ago

#### THE VOYAGE OF THE "VEGA" AROUND ASIA AND EUROPE

By A.E. Nordenskjöld (Macmillan & Co., 1881)

THE voyage of the *Vega* will be in many respects one of the most memorable events in the history of navigation. For the first time a continent has been circumnavigated, so far as authentic record goes, and at last the North-East Passage has been won, after heroic efforts begun nearly three and a half centuries ago. But the voyage will be still more memorable by the two rich volumes in which it finds copious record, volumes which have scarcely a parallel in the whole literature of geographical exploration.

In one chapter, Baron Nordenskjöld has collected all the information attainable on Steller's sea-cow (Rhytina Steller'), which on Steller's visit to Behring Island in 1741 was found pasturing in large herds on the abundant sea-weed on the shores of the island. Twenty-seven years after, not a specimen was to be found, and it was believed to be then

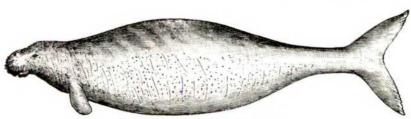


Fig. 10.-Reconstructed form of the sca-cow.

extinct. But Baron Nordenskjöld adduces evidence to prove that a specimen was seen twenty-seven years ago, though there can be little doubt that it has really gone the way of the mammoth. The Baron does not believe that its extinction is due to the destruction by hunters, but that it was a survival from a past age doomed to extinction, which overtook it when driven from its pastures on the shores of Behring Island.

"Steller's sea-cow (Rhytina Stelleri, Cuvier) in a way took the place of the cloven-footed animals among the marine mammalia. The sea-cow was of a dark-brown colour, sometimes varied with white spots or streaks. The thick leathery skin was covered with hair which grew together so as to form an exterior skin, which was full of vermin and resembled the bark of an old oak. The full grown animal was from twenty-eight to thirty-five English fect in length and weighed about sixty-seven cwt. The head was small in proportion to the large thick body, the neck short, the body diminishing rapidly behind. The short fore-leg

terminated abruptly without fingers or nails, but was overgrown with a number of short thickly placed brush-hairs; the hind-leg was replaced by a tail-fin resembling a whale's. The animal wanted teeth, but was instead provided with two masticating plates, one in the gum the other in the under jaw. The udders of the female, which abounded in milk, were placed between the fore-limbs. The flesh and milk resembled those of horned cattle, indeed in Steller's opinion surpassed them. The seacows were almost constantly employed in pasturing on the sea-weed which grew luxuriantly on the coast, moving the head and neck while so doing much in the same way as an ox. While they pastured they showed great voracity, and did not allow themselves to be disturbed in the least by the presence of man. One might even touch them without their being frightened or disturbed. They entertained great attachment to each other, and when one was harpooned the others made incredible attempts to rescue it.'

From Nature 25, 22 & 29 December, 1881.

reassuring in that it showed competent groups to be tracking down environmental nitrosamines, and to be suggesting realistic methods for reducing their formation.

More difficult to elucidate is the formation of nitrosoamines in vivo. Amines are ubiquitous in biological material, and therefore occur in vegetable and animal food. Nitrate occurs in plant material and in water, and can be reduced to nitrite by bacteria in the gastrointestinal tract, especially in the mouth. Hence amines and nitrite are likely to react together in the stomach. The amount of nitrosamines formed depends on the nature of the stomach contents, and has been shown to be altered by gastroduodenal diseases (Walters, Food Research Association, Leatherhead). As nitroso compounds are usually rapidly metabolized, and are formed in small amounts at any one time, their estimation is difficult. An ingenious method is being developed in which the naturally occurring amino acid proline is given to experimental animals or to human subjects. Nitrosation of this amine yields N-nitrosoproline, a nitrosamine which is apparently not carcinogenic and which is excreted in urine. Determination of urinary N-nitrosoproline could therefore give a measure of the formation of N-nitroso compounds in man (Ohshima and Bartsch, International Agency for Research on Cancer, Lyon). Where reduction of the nitrate content of food would lead to other problems, as in the case of cured meat, it is possible to add compounds, such as ascorbic acid, which, in certain conditions, reduce nitrosamine formation (Tannenbaum, MIT).

Many useful drugs are amines which can give rise to carcinogenic nitrosamines. Here the only solution at present seems to be a risk/benefit evaluation with each drug, possibly with each patient. To take cimetidine for dyspepsia, whether or not an ulcer is known to be the cause, or to take this drug indefinitely to prevent recurrence of an ulcer, requires justification.

The levels of nitrosamines to which man is exposed may be low at any one time, but the effect of one nitrosamine given at different times, and of different nitrosamines administered simultaneously, can be additive. Hence to ask each time nitrosamines are detected in a product, "is this level likely to constitute a hazard to man?", is meaningless. The nitrosamines consumed in Scotch whisky must be added to those in bacon, mushrooms, cigarette smoke, cosmetics, and to the nitrosamines formed *in vivo*.

While the entire population is probably exposed to nitrosamines in some form, everyone does not get cancer. The more that is understood about the means by which nitrosamines cause cancer, the more it is possible to detect high-risk individuals or groups, and to take protective measures. The best working hypothesis is that metabolism of nitrosamines leads to the formation of alkylating species which react

with DNA and cause mutation-like events which disrupt certain control mechanisms in the cell.

The first biochemical step in carcinogenesis, the metabolism of the aliphatic, heterocyclic, or aromatic nitrosamines, has been studied by chemical in vivo and in vitro methods (Okada, Tokyo Biochemical Research Institute; Preussmann). Oxidation occurs at various carbon atoms, but it was pointed out that the major metabolites may represent genuine detoxication reactions, while metabolites produced by minor pathways, and which would be more difficult to detect, may represent the ultimate carcinogens (Lijinsky, Frederick Cancer Research Center, USA). The effect of replacing certain hydrogen atoms in the nitrosamine by deuterium, with the consequent slowing of oxidation at this site, on the carcinogenicity of the nitrosamine gives suggestive data on the relevant reactions (Lijinsky). The effect of components of a normal diet, for example alcohol, on metabolism of nitrosamines is obviously of much relevance in assessing the role of

these compounds in human cancer (Hecht, Maylor Vana Institute, Valhalla, USA). Also the occurrence in cooked food of chemicals which increase the mutagenicity of nitroso compounds, and cause previously inactive compounds to become mutagenic, is a cause for concern (Wakabayashi, National Cancer Centre Research Institute, Tokyo).

One in four of the population develop cancer, and much of this is thought to be due to environmental agents (Higginson, International Agency for Research on Cancer, Lyon), which are, at present, mainly unidentified. The universal distribution of nitrosamines and the variety of tumours they are known to induce in animals make it difficult to correlate exposure with any particular human cancer. However, there is now epidemiological evidence that strongly suggests the association of N-nitroso compounds with oesophageal, bladder and stomach cancer. The fact that a variety of preventive measures are being taken to reduce exposure is a cause for special congratulation to those engaged in this work.

#### Movement in membranes

from A.G. Lee

IT is now ten years since Singer and Nicholson unveiled their fluid mosaic model of the biological membrane to an admiring world. To have survived so long is a complement both to the basic correctness of the model and to its flexibility. Predictably, much work over the intervening period has been concerned with trying to reduce this flexibility and achieve a state from which it is possible to make concrete predictions about membranes. Progress has, however, been slow and the gap to be bridged between simple model systems and studies of intact membranes remains large.

One source of difficulty is obvious — the membrane is a complex mixture of components, and one of the keys to success in the molecular sciences is to avoid complex mixtures. In the long run, this problem has to be faced in membrane studies, since an understanding of how the various components of the membrane mix together is one of the central issues of membrane biology. All that we can say with certainty at present is that the components of the membrane will not be mixed randomly. but the exact nature of the resulting order is unclear. Order in the membrane will follow in part from the equilibrium thermodynamic mixing properties of the components of the membrane, but it could also reflect the role of larger-scale structures in

preventing any such equilibrium mixing. The latter possibility is illustrated in a recent study by Dragsten, Blumenthal and Handler (see this issue of *Nature*, p.718).

Epithelial cells form sheets which serve as permeability barriers in organs such as kidney and the intestine. To be effective as a permeability barrier the cells have to be linked together by an elaborate junctional complex, called a tight junction, which forms a continuous, belt-like, highly impermeable structure around the cells. A further property of the cells is that they are asymmetric, with channels, pumps and receptors being different on the apical (mucosal) and basolateral surfaces. It has been suggested that this asymmetry is possible because the tight junction acts as a barrier to lateral diffusion in the membrane. Using the technique of fluorescence photobleaching, Dragsten and his colleagues have shown that cell surface sites in epithelia labelled with lectins do not diffuse laterally at all, so that no barrier is required to maintain an asymmetric distribution of these components. They also found a much more interesting result using lipid labels added to monolayers of epithelial cells. A fluorescently labelled phosphatidylcholine, for example, was free to diffuse in either the apical or basolateral surface (depending on to which side of the monolayer it was added) but was unable to pass through the tight junction from one surface to the other. However, if the epithelial monolayers were dispersed into

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suspensions of single cells by disruption of the tight junction, then phospholipid could freely diffuse over the entire cell surface.

Not all amphipathic molecules are segregated in this way by the tight junction, and some can freely diffuse from one surface of the cell to the other. These differences appear to be correlated with the ability of the label to undergo the so-called 'flip-flop' motion between the two halves of the bilayer membrane. Phospholipids are known to undergo this motion very slowly, and a phospholipid added to epithelial cells remains in the outer half of the membrane. Some amphipathic molecules can, however, rapidly flip between the two halves of the membrane. and it is these labels that can diffuse past the tight junction. It therefore seems that the tight junction acts as a diffusion barrier only for the outer half of the membrane and so allows the epithelial cell to maintain a difference in phospholipid compositions between the outer halves of the apical and basolateral surfaces of the cell.

Restricted diffusion of phospholipids and other amphipathic compounds can also result from the simple equilibrium mixing properties of the components of the membrane. One clear example has been studied by McConnell and his colleagues over the last few years (Recktenwald and McConnell Biochemistry 20, 4505; 1981). There is now much evidence that in mixtures of phospholipids and cholesterol some kind of highly ordered structure is formed. Although the full details are still controversial, the suggestion is that the structure resembles a ploughed field with furrows of pure phospholipid separated by ridges made up of a mixture of phospholipid and cholesterol. Diffusion will then tend to occur preferentially parallel to the ridges and furrows rather than across them. In extrapolating this conclusion to real membranes, many of which contain high concentrations of cholesterol, it is of course necessary to consider the effects that proteins might have. Effects of protein on the diffusion of amphipathic molecules are considered by Laggner in a recent issue of Nature (294, 373; 1981).

If spin-labelled fatty acids are incorporated into membranes at relatively high concentrations, then the ESR spectra of the spin labels become broadened as a result of motion of one spin label relative to its neighbours. Laggner has observed that this concentration broadening is less in membranes of sarcoplasmic reticulum than in lipid bilayers prepared from phospholipids extracted from sarcoplasmic reticulum. He suggested that this could reflect the presence of two phospholipid environments - bulk phospholipid and annular phospholipid adjacent to membrane proteins, with only the fatty acid incorporated into bulk phospholipid being free to diffuse within the membrane. The pattern of diffusion of amphipathic molecules in biological membranes is clearly complex.

# The gassiest comet?

from David W. Hughes

COMET BRADFIELD, 1979X, is the comet in question and recent measurements indicate that the ratio between the mass of gas and the mass of dust emitted by this comet per unit time is higher than that measured for any other comet. The underlying message is that no two comets seem to be the same and when they near the Sun their gas and dust emission depends on a whole range of factors. Among the most important of these must be the time interval between perihelion passages, the orientation of the nucleus spin axis with respect to the orbital plane and the evolutionary stage the comet happens to be in.

Knowledge of gas production has been greatly improved by two factors. First, high-resolution spectra of bright comets can be obtained from the International Ultraviolet Explorer (IUE) satellite. The satellite has two echelle UV spectrographs that have a total wavelength range of 1.150-3,200 Å and a possible resolution of 0.2 Å, over a rectangular field of view of 10×15 arc s. Data have been collected from objects as faint as 17th magnitude. Second, an upsurge in interest in the UV end of the spectrum has helped the groundbased astronomer and it is now realized that reliable, absolute photometry can be carried out from a good site down to wavelengths of around 3,080 Å.

Considerable extinction occurs in the atmosphere at low wavelengths due to Rayleigh scattering by air molecules, aerosol scattering by suspended particles and molecular absorption by ozone. Most of the ozone resides between the altitudes 10 and 35 km and the column density varies with season and latitude. Its contribution to extinction is, for example, 20 per cent smaller at the latitude of Hawaii than at the latitude of Flagstaff, Arizona. Unfortunately the extinction caused by Rayleigh and aerosol scattering varies exponentially as a function of height - for UV observations the higher you are the better. Extinction can, however, be measured by making repeated observations of a standard star as it rises and sets. The results obtained vary from 80 to 20 per cent as the wavelength increases from 3,100 to 4,100 Å.

Ground-based observations of comet Bradfield have been made by Michael F. A'Hearn (University of Maryland), Robert L. Millis (Lowell Observatory, Arizona) and Peter V. Birch (Perth Observatory, Australia) and their results have been published in a recent edition of *The Astronomical Journal* (36, 1559; 1981).

Comet Bradfield came very close to the Earth and although it was not unusually

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luminous, it was possible to make detailed photometric measurements from a time just after its discovery, when it was 0.57 AU from the Sun, up to the time when it was 1.65 AU away. All observations were postperihelion. The unusual apparition geometry meant that the comet quickly changed its position on the celestial sphere and had to be observed progressively from Perth, Australia; Mauna Kea, Hawaii; and Flagstaff, Arizona. Photometric measurements were made of the comet using a series of filters with pass bands of around 100 Å. The coma of the comet contains many gas molecules which undergo fluorescence excitation by solar radiation. Filters centred on 3,085, 3,365, 3,870, 4,060, and 5,115 Å pick up the radiation from OH, NH, CN, C3 and C2 respectively. The dust-scattered continuum was sampled by using filters centred at wavelengths of 3,300, 3,675 and 5,240 Å.

Using a specific coma model the authors converted the observed cometary molecule column density to a value for the total number of molecules in the coma and then, by dividing by the lifetime of the species, to a value for the production rate from the nucleus. To check their work they compared their ground-based observations of the production of OH with those determined from the IUE spacecraft. The agreement was excellent.

The production rate of all molecular species was found to vary as  $r^{-3.2}$  where r is the heliocentric distance of the comet. This is remarkably steep and contrasts with the  $r^{-2}$  variation found for comet West. One suggestion is that the nuclei of the two comets differ. Bradfield is perhaps covered with an outer 'frosting' of relatively volatile material. However, frosting is generally thought to be associated with a long residence  $(10^5-10^7 \text{ yr})$  in the boundary regions between stars, whereas comet Bradfield has an orbital period of only about 300 yr. Maybe the surface of the nucleus is heterogeneous or suffers from the buildup of an insulating crust.

The gas to dust ratio of the comet was found to be higher than for any other comet observed previously. The equivalent widths of certain C<sub>2</sub> Swan bands indicate that this could have been as high as 22 when the comet was about 0.8 AU from the Sun, decreasing to about 3 at 1.65 AU. This is three to four times greater than values observed for previous very gassy comets such as P/Encke and comet Kohler (1977 XIV).

Changes occurred in the relative molecular production rate as a function of heliocentric distance. The comet was CN rich when it was discovered at  $r = 0.57 \,\mathrm{AU}$ . Over the range  $0.6 < r < 1.0 \,\mathrm{AU}$  the abundance ratios were normal and they stayed constant. However, a peak in  $C_2$  production relative to CN occurred around 1 AU and OH and  $C_2$  decreased relative to  $C_3$  and CN as the comet went further away.

# REVIEW ARTICLE

# Long-term growth and cloning of non-transformed lymphocytes

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The diversity of lymphocytes has made it difficult to study the properties of a specific lymphocyte type. Here we describe methods used in long-term culture and cloning of T lymphocytes and discuss the potential uses of these cell lines in immunology.

ONE of the greatest obstacles to the understanding of the functions, regulation, cell biology and secreted products of lymphocytes is the enormous diversity of these cells. This diversity is accounted for by: (1) a series of distinct differentiation lineages, that is, B and T cells and their subpopulations; (2) a heterogeneity of differentiated states (resting, activated, memory and effector cells); and (3) a heterogeneity in the specificity of receptors individually expressed on distinct cells. The number of distinguishable specificities expressed by lymphocyte receptors is probably of the order of  $10^7$ . By taking into account the other two classes of heterogeneity, we may estimate conservatively that  $10^8$  distinct types of lymphocytes may exist among the  $\sim 10^{12}$  such cells in an adult human.

Obviously, the direct study of the properties of any specific type of lymphocyte is virtually impossible in such conditions. Traditionally, immunologists have resorted to methods such as the use of 'polyclonal' stimulants which activate most or all cells of a particular class. These approaches have yielded valuable information on lymphocyte physiology but the extent to which the results obtained reflect the reality of antigen-stimulated lymphocyte function is still uncertain. Sophisticated cell purification procedures such as fluorescence-activated cell sorting and the use of antibody-coated dishes, have made it possible to obtain cell populations of a substantial degree of purity, but these procedures by themselves cannot provide truly homogeneous cell populations. Recently, however, long-term culture and cloning of non-transformed immunocompetent lymphocytes has become possible. Here we review the methods of production of such long-term T-cell lines and the potential value of these lines in cellular immunology.

#### Culturing

It had been possible for some time to maintain functional thymus-dependent (T) lymphocytes in culture and to enrich such cultures with cells specific for a given antigen by repetitive stimulation with that antigen. Using this approach, T lymphocytes specific for protein antigens were propagated for periods of 3 months or longer<sup>1</sup> and cytotoxic T-cell lines maintained for periods >60 days<sup>2</sup>. However, such lines generally failed to expand to any substantial degree. It was the recognition that the growth of lymphocytes depended not only on contact with antigen but also on the presence of certain growth factors<sup>3</sup>, such as T-cell growth factor (TCGF or interleukin-2 (IL-2)), which opened the way to the large-scale growth and cloning of T lymphocytes. Various techniques are now available for developing and cloning long-term T-cell lines. Indeed, T lymphocyte lines of essentially all the major functional classes known, including helpers, amplifiers, cytotoxic cells and suppressors, have been prepared and dramatic results obtained with these lines are now being reported4.

Several different methods can be used to establish T lymphocytes in long-term culture. Perhaps the first, and still most widely

used, is to grow T lymphocytes from immunized donors for several weeks or more in the presence of antigen with exogenous growth factor(s), before attempting to clone them<sup>5</sup>. In general, T cells specific for foreign histocompatibility antigens—cytotoxic T cells and amplifier T cells-are grown with allogeneic irradiated stimulator cells whereas T cells specific for protein antigens are grown with antigen and irradiated spleen cells as a source of antigen-presented cells (APCs). A source of histocompatible APCs is particularly critical for cells displaying 'histocompatibility restriction' (see below). As well as presenting antigen, the added irradiated cells probably supply growth or differentiation factors including, but not necessarily limited to, interleukin-1 (IL-1). Cell growth is often quite vigorous in the presence of antigen, a source of an IL-2-containing growth factor and irradiated spleen cells. Cells grown in this way can often be established in long-term lines although they generally go through a period of 'crisis' when they stop growing or grow only slowly. The nature of crisis is not fully understood, but cells which survive it can be maintained in culture for periods of ≥3 vr (F. Fitch, personal communication). It has also been possible to establish cell lines using antigen and irradiated spleen cells, without the addition of an exogenous source of growth factor<sup>6,7</sup> This approach has been particularly successful in the establishment of helper cell lines. Presumably, in this case, IL-2 is produced by the irradiated spleen cells added to these cultures or perhaps by the line itself.

A second approach to the production of long-term T-cell lines is to grow cells as colonies in soft agar as soon as possible after they have been taken from an immunized donor. In this technique, T lymphocytes are stimulated in an initial suspension culture by the addition of antigen and a source of APC, generally in the form of irradiated spleen cells. After 3 days, these cells are distributed in the upper layer of a two-layer soft agar culture system; antigen is present in the lower layer. Colonies form in the soft agar and may be picked from day 4 to day 8. These colonies are then placed in microtitre culture dishes and grown in the presence of antigen, a source of irradiated APCs and an IL-2-containing growth factor. Lines can be established from such colonies with a relatively high frequency of success.

An alternative approach is to select cells for their functional properties rather than their antigenic specificity. For example, Nabel et al. have obtained cells from various sources (that is, fetal liver, bone marrow, thymus and spleen) and grown them with a series of different irradiated filler cells and growth factor-containing supernatants. By analysing the cultures thus obtained, they were able to produce a series of lines of which some mediated helper function, some suppressor activity and others natural killer (NK) activity.

#### Cloning

Each of these strategies for the establishment of long-term lines should be followed by a cloning technique if a cell population derived from a single progenitor is desired. Indeed, it can be anticipated that the longer the interval between the introduction of cells into culture and the time when cloning is carried out, the more likely it is that an 'unusual' cell may come to dominate a line. Consequently, it is desirable to clone as soon as possible, as in the technique described above for preparing lines from soft agar colonies.

Techniques used to clone long-term cell lines are of three general types. Perhaps the most widely used is limiting dilution cloning, in which the cells are diluted to low density (that is, 0.1 to 10 cells per well) and plated out in microtitre wells. In general, such techniques require the presence of some type of filler cell if the T cells are to grow and, for the approach to be useful, the frequency of wells which show cell growth should conform to that expected from Poisson statistics in the case of a single limiting unit responsible for cell growth. Even so, unless the cloning efficiency is quite high, it is advisable to repeat the cloning step before regarding a line as derived from a single progenitor. A second approach is to carry out soft agar colony formation in conditions where the number of antigen-presenting cells exceeds the number of the cultured cells plated. If the efficiency with which cultured T cells form colonies is high (and cases of 90% or greater have been reported10) then it is likely that progeny from such colonies are, indeed, members of a single clone. A third approach is to select a single T cell from a long-term line by micromanipulation or using a cell sorter and to grow a line from this cell. This is perhaps the most difficult but least ambiguous method.

#### T-cell growth factors

The preparation and study of long-term T-cell lines has produced interesting results. First, it has given great impetus to the study of factors regulating T-cell growth, two of which have been characterized in considerable detail. One of these, IL-1, is a macrophage product which seems to play an important part in the activation of some T cells, possibly by stimulating them to produce a second factor, IL-2 (refs 11, 12). Generally, the ability of IL-1 to stimulate T cells seems to depend on the presence of another stimulating agent, either antigen (or antigen-Ia complex) or mitogenic lectin. IL-1 can be obtained from macrophage tumour lines such as P388D1. Production of IL-1 by P388D1 depends on induction with agents such as phorbol myristate acetate (PMA). The technique of superinduction of P388D1 cells has recently provided sufficient IL-1, a protein molecular weight  $(M_r)$  15,000, for it to be purified to homogeneity<sup>13</sup>. In addition to the role of IL-1 as a T-lymphocyte activating factor (it was originally known as LAF), it seems also to function as an endogenous pyrogen<sup>14</sup> and as a stimulant of firoblast proliferation<sup>15</sup> and, thus, potentially may be responsible for much of the fibrosis seen in immune inflammation.

IL-2, a T-cell product which regulates T-cell growth, seems to act on T lymphocytes after they have been stimulated by antigen or by an antigen-Ia complex<sup>16</sup>. Recent studies<sup>17</sup> indicate that resting T lymphocytes lack membrane receptors for IL-2 but that stimulated T cells (that is, T-cell blasts) bear ~10,000 IL-2 receptors (per cell). The binding of IL-2 to T-cell blasts appears to be critical for their completion of the cell cycle.

IL-2 has been purified both from murine and human sources and from normal lymphoid tissue and tumours. In general, IL-2 production requires stimulation of the cell; both EL-4, a murine T-cell lymphoma, and Jurkat, a human T-cell leukaemia, secrete large amounts of IL-2 on induction with PMA. Human IL-2 is a glycoprotein of  $M_r$  15,000<sup>18</sup>; murine IL-2 has an apparent  $M_r$  of 30,000 by gel filtration<sup>19,20</sup> and 23,000 by SDS-polyacrylamide gel electrophoresis<sup>21</sup>.

One major question concerning the regulation of T-cell growth by IL-2 is whether cells which are sensitive to IL-2 also secrete it, perhaps in different phases of their growth cycle, or whether IL-2 production and IL-2 sensitivity are always properties of independent T-cell lines. There are clear cases of IL-2-dependent lines which do not produce IL-2, for example, the cytotoxic T-cell lines available in many laboratories, while certain lines, such as those which express allogeneic mixed

lymphocyte reactivity, produce IL-2 during growth. However, there has been no clearly documented case of an IL-2-sensitive, non-transformed line which also produces IL-2, although T cell tumours with these properties have been described 17,35.

#### T-cell physiology

Cloned T-cell lines have potentially great value in the study of T-cell physiology. They have already aided our understanding of the specificity of so-called 'histocompatibility-restricted' T cells and of the nature of the immune response (Ir) gene product. In general, cells which mediate helper, amplifier or delayed hypersensitivity functions, which proliferate in vitro to antigen, or which act as cytotoxic effector cells, seem to recognize antigen and a product of the major histocompatibility complex (MHC)<sup>22</sup>. The MHC product recognized in these reactions is often referred to as a restriction element. For helper, amplifier, delayed hypersensitivity and proliferating T cells, the restriction element is generally an Ia antigen. This is a two-chain molecule; genes for both chains are encoded in the I region of the MHC. For cytotoxic T cells, the restriction element is usually an H-2K. D or L antigen. The study of cloned long-term T-cell lines has provided unambiguous evidence that joint recognition of antigen and restriction element is the property of a single cell and not of two collaborating cells, one specific for antigen, the other for the restriction element.

Studies of cloned long-term lines of antigen-specific human T lymphocytes have established that they, too, demonstrate histocompatibility restriction<sup>23</sup>, a matter which had previously been in doubt for human T cells because of the difficulty in obtaining antigen-specific cells of the correct genotype and because the mixed lymphocyte reactions observed in cell mixing experiments gave confusing results.

Cloned mouse T-cell lines have been used to show that in heterozygous  $(F_1)$  individuals, restriction elements may be formed by the pairing of a product encoded by one parental haplotype and a product encoded by the other parental haplotype  $^{24.25}$ . There is chemical evidence of Ia antigens that are created by such genetic complementation  $^{26}$ . Clonal analysis suggests that clones specific for restriction elements created by complementation (heterozygous restriction elements) are only slightly less frequent than those specific for homozygous restriction elements. Indeed, the agreement between formation of both restriction elements and Ia antigens by complementation provides strong evidence that the Ia antigen is the restriction element. Similarly, using cloned T-cell lines to analyse the action of Ir genes, it has been possible to show that the products of these genes are also restriction elements and Ia antigens  $^{24}$ .

Finally, the use of cloned T-cell lines has provided insight into the provocative finding that the frequency of T lymphocytes specific for foreign MHC antigens (that is, capable of mediating mixed lymphocyte responses) is very high. It has been estimated that ~5% of the T lymphocytes of an inbred mouse are specific for the MHC antigens of any single foreign MHC haplotype. It has recently been shown<sup>27</sup> that a cloned cytotoxic T-cell line specific for an H-Y antigen and a self-MHC restriction element could also lyse cells of a specific foreign MHC type that did not express the H-Y antigen. Similarly, a clone of T cells from a mouse of MHC haplotype, H-2<sup>a</sup>, which could be stimulated to proliferate by dinitrophenyl-ovalbumin (DNP-OVA) in the presence of APC-expressing H-2a-encoded restriction elements, could also be stimulated to proliferate by APC-expressing H-2° antigens in the absence of DNP-OVA<sup>28</sup>. Thus, these antigen-reactive H-2<sup>a</sup> cells also mediate a mixed lymphocyte response to H-2s-encoded Ia molecules. Indeed, recent studies<sup>29</sup> suggest that a very large fraction of cloned antigenspecific, MHC-restricted T-cell lines are also responsive to foreign MHC antigens. Thus, it seems likely that a high frequency of alloreactive T cells is observed because alloreactivity may be a property of almost all MHC-restricted T cells. This will be of critical importance in understanding the chemical basis of T-cell antigenic recognition and the evolutionary forces which have shaped the T-cell repertoire.

Recently, the approaches used to grow long-term lines of T cells have also been applied to B cells. Lines of non-transformed, growth-factor dependent B lymphocytes of mouse<sup>30</sup> and human<sup>31</sup> origin have been propagated for periods of >10 months. The growth of these cells seems to depend on a growth factor produced by T lymphocytes but which is distinct from IL-2 (M. Howard et al. and W.E.P., submitted for publication, and B.S., B. Stadler, J. J. Oppenheim and W.E.P., unpublished observations). This factor has been termed B-cell growth factor (BCGF) and studies of its properties are now in progress. B cells grown in long-term culture can be stimulated to secrete immunoglobulin and human B-cell lines have been successfully cloned by limiting dilution.

#### **Potential**

We may anticipate that the use of cloned T-lymphocyte populations will be of great value in the functional, molecular and genetic characterization of T-lymphocyte receptors and will lead

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to a more complete understanding of both the molecular basis and biological significance of histocompatibility restriction. We may also anticipate that a further understanding of the regulatory interactions of immunocompetent cells will come from the preparation of cloned lines of each cell type and from the analysis of the molecules that mediate these interactions. Furthermore, the application of this technology to human T<sup>3,22</sup> and B30 lymphocytes will have great importance in various clinical situations. Cloned T-cell lines will be valuable as tissuetyping reagents<sup>32</sup> and may have applications in specific therapy for certain infectious agents (J. M. Chiller, personal communication) and tumours 33.34

Much work remains to be done; it is particularly important to establish the extent to which such long-term cells are 'normal' (that is, how closely they resemble lymphocytes in vivo). It seems reasonable to predict that long-term lines of cloned lymphocytes will become standard tools of the cellular immunologist.

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# The 1883 eruption of Krakatau

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The 1883 eruption of Krakatau was a modest ignimbrite-forming event. The deposits are primarily coarse-grained dacitic, non-welded ignimbrite. Large explosions produced pyroclastic flows that entered the sea, generating destructive tsunami. Grain-size studies of the ignimbrite suggest that these explosions were not driven by magma-seawater interaction. The total bulk volume of pyroclastic deposits, including co-ignimbrite ash, is estimated to be 18-21 km<sup>3</sup>.

ALTHOUGH the paroxysmal eruption of Krakatau (or Krakatoa) in 1883 was very spectacular there are little data relating to the mechanism of the eruption, or to interpretation of the pyroclastic deposits in terms of modern volcanologic theory. Studies have concentrated on Anak Krakatau, a young cone growing in the 1883 caldera<sup>1</sup>, and not on the 1883 deposits. The idea of caldera formation by explosive removal of cone material has been revived<sup>2</sup> but we dispute this mechanism.

We report here results mainly from new field investigations at Krakatau during September 1979. We attempt to correlate the eruption sequence reported previously with the stratigraphy of the deposits to re-evaluate proposed mechanisms for the eruption.

Most interpretations of the Krakatau event rest on the studies 4. He was the first to propose that the huge explosions were caused by seawater coming into contact with the magma within the volcano. Deposits resulting from such phreatomagmatic activity for other eruptions are described elsewhere<sup>5</sup>. Verbeek also suggested that reaction with seawater caused the magma to froth, thus forming the extensive pumice ejected in the 1883 eruption. Although this latter interpretation is now seen to be incorrect, the question of interaction of the magma with seawater as a possible cause of the catastrophic explosions is still being debated2.6. However, the eruption is usually ascribed to such phreatomagmatic activity.

Later workers have recognized that the near-source deposits of the 1883 eruption were largely emplaced by pyroclastic flows: Williams and McBirney<sup>7</sup> suggested that these flows may have swept for some distance across the floor of the Sunda Straits. We now present evidence that the culminating event of the Krakatau eruption was the generation of pyroclastic flows by gravitational collapse of the eruption column after several

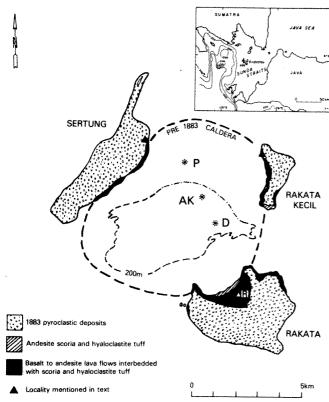


Fig. 1 Sketch map of Krakatau Islands showing prehistoric caldera, possible 1883 caldera outline (200-m isobath, lighter dashed line, dash-dot where conjectural) and simplified geology. R, Rakata cone; P and D are positions of Perbuwatan and Danan vents on pre-1883 Krakatau Island; AK, vent of Anak Krakatau. Outline of islands is from Indonesian Geological Survey map (1940). Inset: location of Krakatau Islands; isobaths in metres (after ref. 2).

large magmatic explosions. We discuss here the role of magmaseawater interaction during the eruption and suggest that the major tsunami that accompanied the eruption were produced when the pyroclastic flows entered the sea.

Before the eruption of 1883, Krakatau consisted of a large island 9 km long and 5 km wide constructed of three volcanoes, Perbuwatan, Danan and Rakata, situated along a fissure within a prehistoric caldera ~7 km in diameter. The surrounding smaller islands of Sertung (formerly Verlaten Island) and Rakata Kecil (Lang Island) are remnants of the rim of the prehistoric caldera (Fig. 1). Krakatau Island largely disappeared during the 1883 eruption, leaving only the southern portion of the cone of Rakata volcano (and a small rock pinnacle) exposed above sea level. The consensus of opinion is that the main vent or vents for the 1883 eruption lay between the vents of Perbuwatan and Danan. Our results are consistent with a vent location in this general area; the location of Anak Krakatau may be controlled by the 1883 vent and conduit.

The foundation of the Krakatau volcano is largely basaltic andesite to andesite composition<sup>9</sup>. The 1883 eruption produced a widespread deposit of non-welded dacitic ignimbrite which covers major portions of the present Rakata, Sertung and Rakata Kecil Islands.

#### Stratigraphy of the 1883 deposits

We have compared the stratigraphy of the pyroclastic deposits examined in the field with the contemporary eruption records compiled by Verbeek<sup>3,4</sup>, and with the later work of Stehn<sup>10</sup> and Williams<sup>8</sup>. The chronology of the eruption, tsunami, stratospheric effects, and the global spread and transport of long residence time 'dust' were documented in the Krakatoa Committee Report of 1888<sup>11</sup>.

A composite section of the pyroclastic deposits of the 1883 eruption is shown in Fig. 2. The stratigraphy of the airfall and

pyroclastic surge beds is based primarily on two localities on Sertung and Rakata Kecil Islands (Fig. 1), and the stratigraphy of the pyroclastic flow deposits (ignimbrite) is based on several cliff exposures on the western coasts of Rakata and Sertung Islands.

The 1883 eruption began with a period of intermittent mild explosive activity on 20 May 1883. Contemporary reports suggest that on 26 August 1883, at about 13h 00min LT the volcano went into an increasingly explosive phase of eruption, producing a more or less continuous eruption column through explosions at brief intervals ( $\sim 10$  min) (see Judd in ref. 11). The largest explosions during this stage of eruption were recorded at 17h 07 min on the evening of 26 August and 1h 42min, 2h 25min and 4h 43min on 27 August (Fig. 3; see Judd in ref. 11). The eruption column is reported to have been up to 25 km high during this interval. Ships within  $\sim 20$  km of the volcano reported heavy ash fall, with large pumice clasts (up to  $\sim 10$  cm diameter) (Judd in ref. 11).

The explosions produced fall units of dacitic pumice up to 20 m thick that are exposed on the islands of Rakata Kecil and Sertung. Pyroclastic surge deposits, found interstratified with the fall units, indicate that minor eruption column collapse during this stage of the eruption produced thin, low-density turbulent pyroclastic surges 12. All outcrops of the fall/surge deposits are very close to the source (only 2 or 3 km from the supposed vent) and it is inferred that the surges were fairly localized (with much material probably flowing onto or into the sea). The fall deposits were probably of sub-plinian type 13,14 and also of modest dispersal. For example, there were reports of only minor ash fall in southern Sumatra and western Java before the climax of the eruption of 27 August (ref. 11, p. 14). No pumice fall deposits were found beneath pyroclastic-flow and surge deposits on Rakata Island, only 6 km south-east of the vent, even though exposure was excellent. However, the axis of dispersal of the ash fall may have been directed to the west or south-west4.

On Rakata Kecil Island, an estimated 3 km from the source vent, two uppermost 5-6-m thick pumice fall units are welded to form a dark-grey coherent deposit, differing markedly in colour from the over- and underlying white dacitic pumice (Fig. 4a). Sparks and Wright<sup>15</sup> have described similar welded airfall tuffs

UNITS	COMPOSITE SECTION	THICKNESS (m)	MAJOR FLOW UNITS	LITHOLOGY
FINE AIR FALL BEDS?		2	?	Fine bedded ash
-	600000000000000000000000000000000000000	3-15	4	Upper flow unit, approx 5m thick on Rakata to 15m thick on Sertung
JCED AFTER Sh 35m 883		10	3	Coarse grained, white - grey
IGNIMBRITE FLOW UNITS PRODUCED AFTER 5h ON 27th AUGUST 1883	00000000000000000000000000000000000000	15-20	2	non-welded ignimbrite, contains pumice blocks up to 1m, juvenile obsidian blocks up to 70cm and lithic blocks in a poorly sorted ash matrix. Pumice is crystal poor (9 wt%) Up to 50m exposed;
SNIMBRITE	00000000000000000000000000000000000000	10	*	Verbeek (ref. 4) reported a total of 80-100m.
_ ×		2-3		Lithic lag fall deposit
STS E		6-7		Pumice fall, incipiently welded in places
SS SS			0-2 NUMEROUS Pyroclastic surge beds, pum	Pyroclastic surge beds, pumice and
AIRFALL PUM AND PYROCL SURGE BEDS DEI FROM 13h ON 2 TO 5h ON 27th A	P(a) 0(0) (0) (1)	5-6 7	FALL UNITS	crystal-rich, cross stratified Pumice fall, incipiently welded Stratified fine and coarse pumice and ash-fall units; up to 12m
PRE-1883	NEW TOTAL			Andesite lava flows

Fig. 2 Composite section through the 1883 pyroclastic deposits. Layers of fine ash at top of section were observed from the boat in cliff sections but were inaccessible to examination in the field.

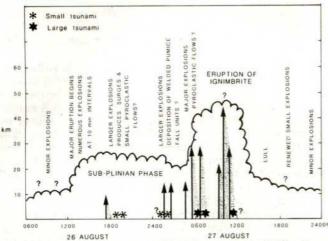


Fig. 3 Sequence of reported explosions and tsunami during 26-27 August 1883 (all times are local). Data from refs 3, 4 and 11. Relative magnitudes of explosions, as recorded by the Batavia gasometer, are indicated by lengths of arrows. Approximate eruption column heights given at left are not accurate to more than ±5 km. Travel times of tsunami from Krakatau to Java and Sumatra coasts estimated at between 30 min and 1 h (ref. 11).

of silicic composition; such tuffs are localized around the vent and indicate high rates of eruption and accumulation of pumice, but imply perhaps only moderate ( $<2\,\mathrm{km}$ ) heights for the gas-thrust part of the eruption column. This combination allows the hot, plastic pumice to sinter and agglutinate under its own weight on deposition. Grain-size analyses of the incipiently welded fall unit show it to be rather poorly sorted ( $\sigma\phi = 3.2$ ), and similar to many previously studied non-welded proximal pumice-fall deposits (Fig. 5).

That the pumice-fall deposits are relatively coarse grained compared with phreatoplinian<sup>5</sup> deposits (Fig. 5) suggests that, during the sub-plinian phase of the eruption, the Krakatau explosions were largely magmatic and did not involve significant interaction with seawater. The lithic-poor nature of most of the fall deposits also suggests that, during these early stages of the eruption, large-scale break-up and collapse of the volcanic edifice into the conduit (to allow access of seawater) did not occur. The surge deposits at Krakatau are also lithic poor and similar to the thin pumice-rich ground-surge deposits associated with other ignimbrite-forming eruptions and described from several other localities<sup>16</sup>.

At about 5h 30min on 27 August, the style of the eruption changed dramatically (Fig. 3). The first of five enormous explosions took place. Wharton<sup>11</sup> gives the times of the largest explosions as 5h 30min, 6h 44min, 9h 29min, 10h 02min and 10h 52min LT on 27 August; Verbeek<sup>3,4</sup> gives slightly different times (3–5 min later) based on a different reading of the gasometer tracing from Batavia, which fortuitously recorded the air waves from the explosions.

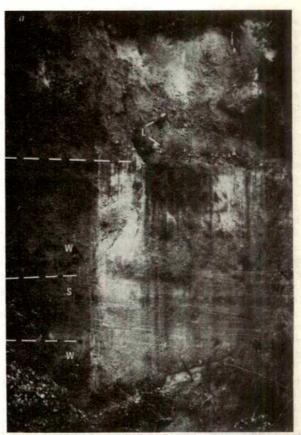
We suggest that each large explosion yielded a pyroclastic flow, almost instantaneously, by large-scale gravitational collapse of the eruption column. These flows moved rapidly off the island and into the sea. The four thick major pyroclastic flow units observed in the field (Fig. 2) probably correspond to the four largest explosions (Fig. 3; see ref. 10).

Material came from the vent between explosions, but was probably finer grained and not of significant volume. We may compare this with the Mt Ngauruhoe, New Zealand eruption sequence of 19 February 1975 (ref. 17) where after an initial period of more or less continuous magma flux, activity declined, then began again with a series of large explosions, each producing a small pyroclastic flow. We propose that the Krakatau eruption sequence was similar but on a much larger scale.

#### Characteristics of the 1883 ignimbrite

The Krakatau pyroclastic flows apparently moved preferentially to the north and north-east and covered the islands and surrounding sea floor with dacitic ignimbrite (Fig. 6). The biased distribution could be due to directed explosions and/or the topographical control of Rakata volcano (813 m) on the collapsing eruption column, forcing material northwards. The only reports of burns caused by hot ash and gases come from the area around Kalimbang in southern Sumatra, 40 km north-east of Krakatau and 25 km in a direct line from temporary islands produced by the pyroclastic flows. The destruction to the north may have been at the outer edge of a directed blast zone, or perhaps due to ash-cloud surges generated off the tops of moving pyroclastic flows. Such ash-cloud surges can apparently move across water as they did in the St Pierre disaster in 1902<sup>18</sup>.

Deposition of ignimbrite extended Sertung and Rakata Kecil Islands and the southern and eastern parts of Rakata (Fig. 6). Shallowing of the sea floor as far as 15 km to the north of Krakatau<sup>3</sup> was caused by submarine pyroclastic flows that



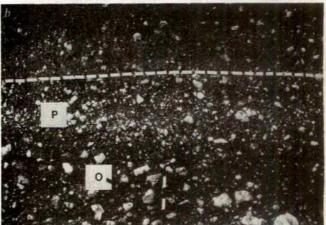


Fig. 4 a, Welded pumice (W), pyroclastic surge (S) and lag (L) deposits on Rakata Kecil Island at locality shown in Fig. 1. Cliff section, 15–18 m high. Above and between lag layer is 1883 ignimbrite. b, 1883 Ignimbrite exposed on Rakata Island. Dashed lines, flow-unit boundary; P, indicates pumice concentration at top of flow unit; O, juvenile obsidian clast. Scale is 50 cm long with 10 cm bars.

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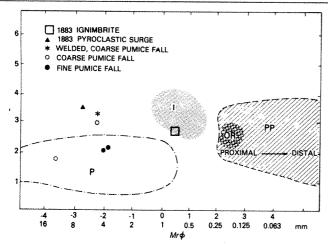


Fig. 5 Grain-size characteristics of Krakatau 1883 pyroclastic deposits, summarized on a plot of median diameter  $Md\phi$  (=  $\phi_{50}$ ) against graphical  $\sigma\phi$  (=  $\sigma_{84} - \sigma_{16}/2$ ), a sorting parameter. The Krakatau pumice-fall deposits are compared with the field of proximal plinian fall deposits (P) and the field of phreatoplinian deposits (PP), after ref. 5. The Krakatau ignimbrite (plot shows area of four analyses) is compared with the field of normal subaerial ignimbrites (layer 2b in ref. 16), (I) and that of ignimbrite produced from a phreatomagmatic eruption, the Oruanui (Or) ignimbrite (S.S., in preparation).

largely filled the 30–40-m deep basin in the sea floor; two new temporary islands, Steers and Calmeyer Islands, were portions of the  $\sim$ 40-m thick ignimbrite exposed above sea level (Fig. 6). Judd (ref. 11, p. 28) erroneously attributed these pumice banks to the growth of parasitic cones on the flanks of the Krakatau volcano.

Yokoyama<sup>2</sup> suggested that these temporary islands were composed primarily of lithic material derived from the explosive removal of the missing portions of Krakatau Island. However, the field descriptions of Verbeek<sup>3,4</sup> and his chromolithographs of these islands<sup>4</sup> clearly show them to be composed of light-coloured pumice and ash similar to the ignimbrite exposed at Krakatau. The >15 km 'run out' distance of the pyroclastic flows was a result of the vast momentum of several km<sup>3</sup> of pyroclastic debris collapsing from a height of perhaps >5 km (ref. 19).

The 1883 magma was a sparsely porphyritic dacite with  $\sim 65-68$  wt % SiO<sub>2</sub>; the small phenocrysts are plagioclase, augite and minor opaques<sup>4,9</sup>. A small amount (< 5 wt %) of grey and white-streaked, mixed pumice is also present.

The ignimbrite is composed of non-welded pumice and ash with  $\sim 5$  wt % lithic fragments (Figs 2 and 4b). A small but significant component (< 5 wt % of the deposit) is non-vesiculated to poorly vesiculated obsidian that we consider to be juvenile. The Krakatau ignimbrite has the typical grain-size characteristics of a sub-aerially erupted ignimbrite (Fig. 5). This contrasts with fine-grained phreatoplinian deposits and associated ignimbrites (S.S., in preparation), which were apparently erupted through water. These ignimbrites are much finer grained than the Krakatau ignimbrite, especially in their lack of large-sized components near source (Fig. 5). This again indicates that magma—seawater interaction did not have a significant role in the fragmentation of the magma during the 1883 Krakatau eruption.

#### Initiation of ignimbrite-forming explosions

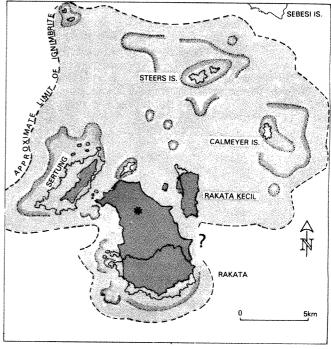
Verbeek<sup>3,4</sup> suggested that the Krakatau eruption became submarine at about 10h on 27 August as a result of foundering of the volcanic edifice, and that the largest explosions were caused by seawater rushing into the vent, making contact with the hot magma and causing it to froth explosively into pumice. By contrast, Judd (in ref. 11) argued that entry of large amounts of seawater into the vent would chill the magma and cause it to crust over. Gas would then accumulate below this crust until explosive pressures were reached. These ideas both assume that

the volcano was collapsing before the explosions. However, the evidence suggests that this was not the case, and that the subsidence at Krakatau and formation of the caldera took place late in the eruption sequence.

First, lithic debris makes up only  $\sim 5\%$  of the deposits which suggests that large-scale collapse of old cone material into the vent was not taking place during the eruption. Second, the coarse-grained nature of both the pumice-fall deposits and the ignimbrite suggests that fragmentation was not caused by magma-seawater interaction. Third, the pyroclastic flow deposits that cover the southeastern part of Rakata Island, and which must have come from one of the vents to the north-west, would be cut off from their apparent source by the caldera wall collapse (Fig. 6). Thus we propose that the large pyroclastic flows were erupted before major collapse of the volcano.

The 1883 caldera lies within the prehistoric Krakatau caldera ( $\sim$ 7 km diameter; Fig. 1), and seems to be a graben-like feature extending along two intersecting zones of fractures<sup>8</sup>. The 'missing' portion of the cone of Rakata may represent a large-scale slump of material into the east-west elongated caldera. The >250-m deep caldera was apparently not extensively filled with deposits of 1883 ignimbrite, also suggesting caldera collapse after the eruption of the major pyroclastic flows and emptying of the magma chamber.

We propose that seawater in large quantities did not gain access to the vent during the most explosive stages of the eruption but that seawater may have leaked slowly into the conduit area, sparking small phreatomagmatic explosions. Such explosions would have broken a cap of viscous magma and allowed sudden, explosive release of large batches of vesiculated magma from beneath the upper conduit system (see refs 20-22). Contemporary accounts indicate that the explosive activity subsided in the early hours of 27 August before the large explosions started (Fig. 3). This may have allowed a partly solidified plug to develop in the vent, facilitating the above



PRE-1883 ISLAND OUTLINE
POST-1883 ISLAND OUTLINES
"SAND" BANKS AND TEMPORARY ISLANDS
SUBACUEOUS IGNIMBRITE
# APPROX 1883 VENT POSITION

Fig. 6 Submarine distribution of the 1883 ignimbrite, inferred from maps and charts in refs 4 and 11. Hatched area shows outline of the Krakatau Islands before 1883. Stippled pattern shows extent of ignimbrite. Also shown are temporary islands and shallow banks where ignimbrite protruded above sea level (outline of islands after ref. 11).

mechanism. The presence of juvenile obsidian clasts in the ignimbrite might indicate partly solidified magma in the vent.

It has been suggested that some large volcanic explosions can be triggered by magma mixing<sup>23,24</sup>. Sudden mixing of rhyolitic and basaltic or andesitic magma might lead to violent exsolution of dissolved volatiles. A small percentage (<5%) of grey and white-streaked pumice occurs in the Krakatau ignimbrite, suggesting some mixing of magmas. However, we cannot envision a magma-mixing process that would adequately explain the sequence and timing of the large explosions of Krakatau as alluded to by Rice<sup>25</sup>. Magma mixing may have had a role in initiating the eruption, but we do not believe that mixing events were the prime cause of the major explosions on 27 August.

#### Co-ignimbrite ash

During the eruption of the pyroclastic flows, much vitric dust was dispersed in the atmosphere and formed a widespread thin, fine ash-fall deposit<sup>4</sup>. Considerable distal ash fall did not occur until the large explosions of 27 August, and we suggest that the widespread ash fall was mainly co-ignimbrite ash<sup>26</sup>.

Mechanisms envisaged for the generation of the high-altitude column of fine vitric dust and gases that accompanies many events involving production of pyroclastic flows are: (1) rise of selectively fine (low terminal fall velocity) material above the vent; (2) generation of ash from the moving pyroclastic flows; and (3) secondary explosions that may occur as hot pyroclastic flows enter the sea<sup>6</sup>. Evidence for this type of secondary explosion was not found at Krakatau, possibly because of the lack of lateral exposure. However, the expected airfall layers produced by such secondary explosions are not present between the ignimbrite flow units at Krakatau in the outcrops we examined.

All large ignimbrite eruptions are accompanied by the production of co-ignimbrite ash<sup>26</sup>. This fine ash consists of the vitric component—pumice shards and fragmented shards commonly from 50 µm down to a few µm in diameter. The finest ash was capable of significant stratospheric residence times varying from days to months (depending on size). This dust may have been partly responsible for the widespread atmospheric optical phenomena observed after the August 1883 eruption (refs 27, 28, Russell and Archibald in ref. 11), although the sulphate aerosols generated by the eruption were probably more important<sup>29</sup>.

#### Volume of the 1883 eruption

Calculations of the total volume of ejecta from the 1883 eruption must include the volume of widely dispersed dust and ash as well as the subaqueous ignimbrite. Volumetric estimates range widely from Verbeek's  $18 \text{ km}^3$  for the total ejecta<sup>3,4</sup>, to the Royal Society's value of  $14.4 \text{ km}^3$  for the fine distal ash fall alone<sup>11</sup>, to recent estimates of  $13 \pm 4 \text{ km}^3$  (ref. 2) and only  $5 \text{ km}^3$  (ref. 30) for the total ejecta based on the volume of the caldera.

Close to Krakatau, Verbeek4 estimated from field investigations and depth soundings of the sea bottom before and after the eruption that 12 km<sup>3</sup> (bulk volume) was ejected. This is a reasonable figure for the volume of ignimbrite produced, based on a sheet of ignimbrite averaging ~ 40 m thick and covering an area of  $\sim 300 \text{ km}^2$  (Fig. 6). Verbeek also considered the volume of widely dispersed ash and estimated this to be 6 km<sup>3</sup>. This value must be a minimum because it only considers a portion of the total dispersal area, which the Krakatoa Committee estimated to be  $1.1 \times 10^6$  miles<sup>2</sup> (see ref. 11, p. 448). Our recalculation of the volume of widely dispersed ash from data in ref. 4, using an area against thickness plot and extrapolating to 1 mm thickness<sup>31</sup>, gives 8.5 km<sup>3</sup>. Recent studies of co-ignimbrite ash volumes compared with the volume of the parent ignimbrite indicate that up to 50% of the magma erupted can be co-ignimbrite ash<sup>26,32</sup>. Applying the methods for estimating crystal concentration in ignimbrites<sup>26,33</sup>, preliminary studies suggest that the Krakatau ignimbrite must have lost at least 40% of the vitric component into the fine co-ignimbrite ash, which therefore has a minimum volume of  $\sim 5~\text{km}^3$ . The above calculations suggest a bulk volume of fine, dispersed ash between  $\sim 5~\text{km}^3$  and  $8.5~\text{km}^3$ .

The pre-27 August sub-plinian fall and surge deposits are thought to be of small volume, probably  $<1~\rm km^3$ . Analysis of samples suggests that only 8% of fine vitric material in these deposits is missing. Therefore, the volume of fine ash produced during the sub-plinian phase of the eruption is very small compared with that emitted during the ignimbrite-forming phase. When the amounts of ignimbrite, co-ignimbrite ash and sub-plinian deposits are added up, the total bulk volume of the 1883 Krakatau deposits is  $18-21~\rm km^3$ . The equivalent volume of dense rock may be roughly estimated as  $9-10~\rm km^3$ .

#### Generation of tsunami

The Krakatau eruption also produced tsunami that inundated coastal areas around the Sunda Straits. These tsunami are generally attributed to submarine explosions or to caldera collapse<sup>8,34</sup> although ejecta falling into the sea has also been suggested<sup>3,4,11</sup>. The evidence indicates that the tsunami were caused by several cubic kilometres of pyroclastic flow material entering into the sea immediately after each of the large explosions.

The early small explosions of 26 August were followed by small tsunami (Fig. 3). For example, an explosion occurred at about 17h 20min on 26 August, and the first destructive waves reached the Java and Sumatra shores 40 km from Krakatau soon afterwards, between 18h and 19h. This early wave or waves may have been due to surges or small pyroclastic flows entering the sea. No other destructive waves were observed until the morning of 27 August (Wharton, in ref. 11). At about 6h 30min a large wave swept over much of the Java coast; another wave followed at about 7h 30min. At about the same time a wave hit low-lying areas of Sumatra. These waves followed the large explosions at 5h 30min and 6h 44min (Fig. 3).

At some time after 10h a gigantic wave (or waves) inundated the coasts bordering the Sunda Straits. This wave is reported to have been the largest, and was the last recorded in the straits; most survivors of the earlier waves had by this time fled from the coastal areas. The tide gauge at Batavia (Jakarta) also recorded waves that correspond to the large explosions.

The sequence and timing of tsunami suggest that the major tsunami were generated at the times of the explosions (Wharton, in ref. 11). Pyroclastic flows resulting from column collapse would have entered the sea within about  $\sim 30 \, \mathrm{s}$  of the explosions  $^{19}$  and initiated the tsunami. The slumping of large parts of the volcano, for example the segment of Rakata cone that probably slid into the caldera, could have been a factor in generating tsunami late in the eruption.

The idea that tsunami can be caused by pyroclastic flows entering the sea is supported by a study of the huge eruption of Tambora in 1815 during which tsunami were generated which flooded the nearby coasts of Sumbawa to a height of 4 m, and Java to a height of 2 m (ref. 33). These waves could not have been produced by magma-seawater interactions or caldera collapse, as the volcanic crater lies some 15-20 km inland at an elevation of about 2,850 m. Pyroclastic-flow deposits from the 1815 eruption entered the sea at the base of the volcano (M.R.R. and S.S., in preparation), and this was the most likely cause. The large tsunami that are inferred to have accompanied the Minoan eruption of Santorini<sup>34</sup> may have been generated in the same way.

We conclude that the 1883 eruption was an ignimbrite-forming event of modest volume. The deposits are largely coarse-grained non-welded dacitic ignimbrite and show little support for a phreatomagmatic origin. The occurrence of welded pumice-fall deposits beneath the ignimbrite also seems to argue against water-magma interaction in the early stages of the eruption. The presence of mixed pumice clasts indicates some magma mixing and supports the contention that welded airfall deposits result, in some cases, from superheating of

magma during a mixing event<sup>15</sup>.

During the paroxysmal phase, four or five large-volume explosions driven by magmatic gases led to collapse of the eruption column, and to the production of pyroclastic flows that entered the sea. The blasts and ensuing pyroclastic flows seem to have been directed preferentially towards the north and northeast. The times of the explosions correlate with the times of tsunami generation, and we propose that the entry of the pyroclastic flows into the sea caused the major tsunami. Caldera collapse probably came late in the eruption.

The total volume of the deposits of the Krakatau eruption is estimated to be 18-21 km<sup>3</sup> (bulk volume), including 12 km<sup>3</sup> of

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ignimbrite and up to 8.5 km<sup>3</sup> of distal co-ignimbrite ash fall. When compared with prehistoric ignimbrite-forming events, ranging in volume up to  $10^3 \, \text{km}^3$  (refs 35, 36), the volume of the Krakatau eruption was very modest.

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# **Evaluation of the Seasat wind scatterometer**

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Surface wind velocities have been derived from backscatter measurements of the ocean surface made by a satellite-borne, microwave sensor. Comparisons with high-quality surface-based measurements obtained during the Joint Air-Sea Interaction experiment are described. The accuracy of the scatterometer winds at this mid-latitude site,  $\pm 1.6$  m s<sup>-1</sup> in speed and  $\pm 18^{\circ}$  in direction, for winds between 3 and 16 m s<sup>-1</sup> is within the design specification.

THE Seasat-A Satellite Scatterometer (SASS) was one of several microwave remote sensors onboard the first oceanographic satellite<sup>1</sup>, Seasat-A, and provided wind velocity estimates over the world's oceans from a height of 800 km. The technique is based on the sensitivity of microwave radar backscatter to the amplitude of short gravity waves (few centimetres) created on the sea-surface by the action of the wind. The sensor and the algorithms used to convert radar backscatter into winds are detailed elsewhere<sup>2-5</sup>

Design specifications for the SASS required a wind speed measurement range of  $4-26 \text{ m s}^{-1}$  with an accuracy of  $\pm 2 \text{ m s}^{-1}$ or 10% (whichever is the greater) and a wind direction range of  $0-360^{\circ}$  with an accuracy of  $\pm 20^{\circ}$ . To check whether these criteria are satisfied the inferred wind vectors must be compared with those obtained by surface instrumentation. Because the accuracy and coverage of routine wind reports at sea are generally not high enough to evaluate the scatterometer measurements properly, a special series of measurements from ships and buoys was conducted in the Gulf of Alaska<sup>6</sup>. It was also fortunate that the field phase of the Joint Air-Sea Interaction (JASIN) experiment took place during the lifetime of Seasat. One of its aims was to provide accurate estimates of the surface meteorological fields over a 200 × 200 km area, and, as a result of careful intercalibrations, high-quality surface wind data are therefore available. This article describes the results of comparisons made at the Seasat-JASIN workshop<sup>8</sup> and demonstrates that the specifications for the SASS have been met for wind speeds up to 16 m s<sup>-1</sup>.

#### Seasat scatterometer

The strength of the radar backscatter (normalized radar crosssection  $\sigma^{\circ}$ ) is a function of the amplitude of very short gravity waves which is itself proportional to the near-surface wind speed. Further, the radar backscatter is anisotropic, so that wind direction can be derived from scatterometer measurements at different azimuths.

The SASS was designed to view the ocean at two azimuths, as shown in Fig. 1. Four dual-polarized, fan-beam antennas were aligned such that they pointed  $\pm 45^{\circ}$  and  $\pm 135^{\circ}$  in azimuth relative to the subsatellite track to produce an X-shaped pattern of illumination on the Earth. In this way, a given surface location was viewed first by the forward antenna and then  $\sim 1-3$  min later (depending on whether the location was at the inner or outer portion of the swath) it was viewed orthogonally by the aft beam. Twelve Doppler filters were used to subdivide the antenna footprint electronically into resolution cells ~15 km (across beam) ×70 km (along beam). In the results presented here pairs of cells were selected having separations <37 km. It is assumed that during the interval between samples the short gravity-wave field integrated over such areas remains constant. The incidence

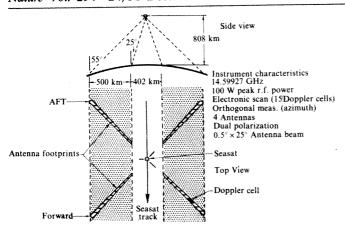


Fig. 1 The swath geometry of the Seasat Scatterometer: viewed from the side (upper) and from the top (lower).

angles of the cells ranged from 25° to 55° for the region of forward and aft beam overlap. Three additional measurements from incidence angles near nadir covered the subtrack, although no directional information can be obtained in this case.

The SASS geophysical algorithm<sup>3</sup> enables wind speed and direction at the sea surface to be computed from  $\sigma^{\circ}$  measurements after correction for attenuation through the atmosphere. This arises principally from water vapour, the vertical integral of which was estimated using data collected by a microwave radiometer on the same satellite<sup>9</sup>.

The model function  $F(W, \chi)$  is the empirical relationship used to describe the dependence of the ocean  $\sigma^{\circ}$  on the wind vector

$$F(W, \chi) = G(\theta_i, \phi, \varepsilon) + H(\theta_i, \phi, \varepsilon) \log W$$

where  $\theta_i$  is the incidence angle,  $\phi$  is the azimuth angle between the wind direction and the radar look angle,  $\varepsilon$  is the polarization of the beam and W,  $\chi$  are the wind speed and direction. Several model functions have been used in the past having different values of G and H for the various combinations of  $\theta_i$ ,  $\phi$  and  $\varepsilon$ . The coefficients were obtained from near-simultaneous measurements of  $\sigma^{\circ}$  and surface wind speed and direction. An airborne scatterometer<sup>10,11</sup> provided 3,000  $\sigma^{\circ}$  values and 2,000 more resulted from SASS during GOASEX6. A least-squares method is used to find values of W and  $\chi$  that, for a given model function, will produce the best fit to the  $\sigma^{\circ}$  observations. Two to four wind vectors result which are approximately equal in magnitude but are widely separated in direction. These multiple local minima (alíases) are due to the harmonic dependence of  $\sigma^{c}$ on wind direction. The solutions are not random and it has been shown<sup>12</sup> that synoptic meteorologists can, using SASS data alone, select the direction closest to the observed wind on a high proportion of occasions. At the time of the Seasat-JASIN workshop the SASS data still contained the aliases so statistics were computed using the solution which gave closest agreement with observations.

Before the workshop, two model functions (W-7 and CWK)

Table 1 Summary of statistics for SASS compared with surface wind field data averaged over all incidence angles

***************************************		CWK		
•	H	V	Both	
Speed (m s <sup>-1</sup> )	$-0.44 \pm 1.58$	$0.50 \pm 1.26$	$-0.04 \pm 1.41$	
Direction (°)	$1.2 \pm 17.9$	$3.4 \pm 15.6$	$0.9 \pm 17.1$	
No.	317	336	704	
		W-7		
***************************************	Н	v	Both	
Speed (m s <sup>-1</sup> )	$0.40 \pm 1.61$	$0.84 \pm 1.60$	$0.62 \pm 1.55$	
Direction (°)	$-0.4 \pm 18.5$	$1.5 \pm 16.6$	$-0.6 \pm 17.1$	
No.	317	336	704	

Statistics for both model functions and different polarizations are shown.

had been developed using the aircraft and GOASEX data sets and were used to generate two sets of SASS wind data for 23 overpasses, chosen to give good spatial coverage of the JASIN area and as wide a range of wind speeds as possible. The JASIN surface data were withheld from algorithm developers and thereby provided the first independent assessment of the scatterometer's wind-measuring capabilities.

#### The JASIN experiment

During JASIN, 14 ships, 3 aircraft and 35 surface and subsurface moorings were deployed in an area 200 km off north-west Scotland during July to September 1978 and several of these platforms were equipped with wind-measuring devices. Three ships, stationed at the vertices of a 200-km triangle, reported winds every hour with a fourth ship at the centre for some of the time. Two other roving ships and several buoys near two of the corners carried data logging systems. Careful intercomparisons were made between the ships and buoys to produce an internally-consistent data set in which interplatform differences in winds are <0.5 m s<sup>-1</sup> and 5°. The absolute accuracy of the winds is difficult to ascertain but both wind-tunnel calibrations and momentum flux comparisons with aircraft suggest that this error is 2 or 3%.

Wind speeds experienced during the 23 Seasat overpasses ranged from 0 to 16 m s<sup>-1</sup> with 83% distributed between 6 and 14 m s<sup>-1</sup>. Measurements were made at heights from 2.5 to 23 m above the surface and in different atmospheric stabilities. In this layer there is considerable variation of speed with height due to friction (direction being constant) so all measurements were corrected to give the 19.5-m neutral stability wind. This is the wind speed that would result at 19.5 m from a given surface stress if the atmosphere were neutrally stratified with an adiabatic lapse rate. The SASS algorithm also outputs the 19.5-m neutral stability wind as it has no means of estimating the stability.

# Comparison of SASS winds with surface-based data

First, SASS winds were compared with autologged data from ships and buoys within the JASIN triangle. The latter provided

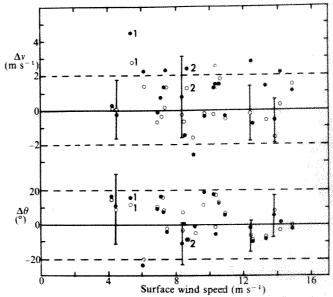


Fig. 2 SASS wind minus surface wind (speed  $\Delta v$  and direction  $\Delta \theta$ ) as a function of surface wind speed. The surface winds are 60-min averages of logged data centred on the time of the satellite pass. All winds have been corrected to 19.5 m. Each point represents the mean difference for all comparisons on a particular overpass (total of 1,150 on the 23 passes). W-7 model; O, CWK. In both cases vertical and horizontal polarizations have been combined. Selected standard deviations are shown for clarity; two cases had much higher standard deviations (>3.5 m s<sup>-1</sup>) and are annotated.

time series of 1-15-min means which were combined to form 60-min means centred on the time at which the satellite pass occurred. This procedure is an attempt to obtain winds which are consistent with the areal average of the SASS. Figure 2 shows differences between SASS and surface winds for both model functions expressed as a function of wind speed. Most of the points lie within the design specifications. The two numbered points refer to different occasions when the differences exhibited large scatter. The more obvious was Case 1 with a mean difference of 4.5 m s<sup>-1</sup> and a standard deviation of 4.7 m s<sup>-1</sup>. For this case, very high SASS winds were indicated having values up to 22 m s<sup>-1</sup> in a 4-5 m s<sup>-1</sup> field. The anomaly was localized and from ship reports and data from the microwave radiometer subsequently identified with a thunderstorm<sup>13</sup>. For Case 2 the surface data revealed that the comparisons were made in an area of horizontal wind gradient ahead of a cold front, which accounts for much of the observed differences, the SASS footprints being some distance from surface platforms.

It is reasonable to assume that in all cases some of the scatter is due to real spatial variability in the winds which is not satisfactorily removed by time-averaging the surface data. An attempt to smooth such effects out was made by constructing wind fields showing isotachs and streamlines based not only on

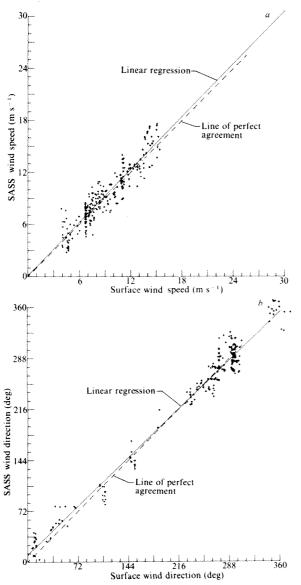


Fig. 3 Scatter plot of: a, SASS wind speed versus surface wind speed for CWK model; b, SASS wind direction versus surface wind direction. The surface data are taken from smoothed fields based on autologged data and manual observations. Slopes: 1.0195 (a); 0.9564 (b). Intercepts: 0.1094 (a); 10.0360 (b). Correlation coefficients: 0.9200 (a); 0.9872 (b).

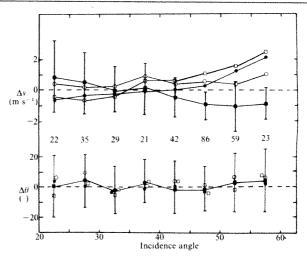


Fig. 4 Comparison of SASS with surface winds derived from plotted fields for various ranges of incidence angle. Horizontal and vertical polarizations for the two model functions are shown. For the CWK, H-pol case the standard deviation of the individual comparisons is also shown together with the number of measurements in each category. Those for the other cases are similar ■, CWK, H-pol; ○, CWK, V-pol; ●, W-7, H-pol; □, W-7, V-pol.

the autologged data but on hourly manual observations from the corner ships to increase the spatial coverage. Values of winds were interpolated at  $0.5^{\circ}$  latitude and longitude grid points and were available for colocation with SASS data points. A crosscheck of these data with the *in situ* observations discussed previously showed agreement to  $\pm 0.6$  m s<sup>-1</sup> and  $\pm 4^{\circ}$ .

Statistics were again calculated using the solution with wind direction closest to the observed value. Scatter diagrams of SASS wind speed and direction (CWK model only) versus surface measurements are shown in Fig. 3 and their dependence on incidence angle and polarization is shown for both W-7 and CWK model functions in Fig. 4. For several categories the standard deviation slightly exceeds the design specification; however, note that for these cases the statistical uncertainty is large because of the limited sample size. These figures illustrate the differences between SASS and JASIN smoothed winds, only a small part of which can be attributed to errors in the JASIN data.

#### **Conclusions**

One objective of the Seasat–JASIN workshop was to demonstrate that winds near the sea surface could be inferred to an accuracy of  $\pm 2~{\rm m~s^{-1}}$  in speed and  $\pm 20^{\circ}$  in direction by means of SASS without prior knowledge of the surface data. In wind speeds up to  $16~{\rm m~s^{-1}}$  the SASS wind vector algorithm recovers wind speed to an accuracy of  $\pm 1.6~{\rm m~s^{-1}}$  for W-7,  $\pm 1.4~{\rm m~s^{-1}}$  for CWK and wind direction to an accuracy of  $\pm 18^{\circ}$  for both model functions (see summary in Table 1). Differences between the two are small and are most noticeable in the dependence of SASS wind speed on incidence angle. The worst cases occur with incidence angles below 25° and above 55° as also found by Halberstam in a comparison with the combined GOASEX/JASIN surface data sets.

In this study the surface data were far superior in accuracy and sampling to data provided by the routine meteorological network. They were also of higher quality than data from GOASEX in that careful analysis of the many intercomparisons between JASIN platforms resulted in reduction of the biases which were present. This in turn has reduced the scatter in comparisons with SASS<sup>15,16</sup>. The scatterometer data are now being used in combination with the JASIN data to increase our understanding of how the atmosphere and ocean interact on a variety of time and space scales.

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# Pontnewydd Cave in Wales—a new Middle Pleistocene hominid site

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An Acheulian industry in association with a hominid molar has been found at Pontnewydd Cave. This tooth represents the oldest hominid specimen known from Wales and, except for the Swanscombe fossil, from Britain. The molar is probably from a young adult and the tooth closely resembles those of 'Early Neanderthal' fossils from Krapina, Yugoslavia, a comparison accentuated by the degree of taurodontism at both sites. Uranium-thorium and thermoluminescent dates suggest an age for the specimen of around 200 kyr. Uranium relative dating indicates a Pleistocene age for two additional unstratified hominid fragments. Sedimentological study of the deposits and examination of the rock types in the cave, both natural and artefactual, indicates earlier glacial activity.

PONTNEWYDD CAVE has been the subject of four seasons of excavation (directed by H. S. Green for the National Museum of Wales). Excavation of the cave in the 1870s by Boyd Dawkins 1.2 and by McKenny Hughes<sup>3,4</sup> produced fauna, including a possible human tooth, since lost, and artefacts which were compared with finds from Le Moustier and St Acheul. No further work took place at the cave until the present excavation except for construction work at its mouth during the Second World War, when the cave was converted to a store.

#### Location and geomorphological setting

The cave is situated in the lower Elwy valley of North Wales (Fig. 1), the entrance opening off the Carboniferous Limestone scarp that forms one side of the valley. The cave entrance is at a height of 89.5 m OD, exactly 50 m above the river Elwy. The valley contains thick deposits of drift, in places built into a series of terraces whose upper surface locally approximates to the height of the cave. The data from the cave should elucidate the Quaternary geology and geomorphology of the area for which. hitherto, there has been no reliable chronological framework in which to place the Quaternary sequence. Moreover, as the cave is situated close to the probable maximum limit of penetration of Irish Sea Ice into the area, it may provide a means of dating the advances of this ice sheet as well as those of the more local Welsh Ice.

#### Stratigraphy

Sedimentological studies were originally aimed to correlate the sequence of the three separate areas of the cave in which substantial deposits survive intact (Fig. 2a: South Fissure and adjacent Deep Sounding; South Passage; and East Passage). High probability correlation, based on lithologic characters, has resulted in the composite stratigraphy shown in Fig. 2b. Interpretation has proceeded using data derived from both standard sedimentological analyses and the study of microtextural assemblages of quartzitic particles by scanning electron microscopy (SEM).

The lowest stratigraphical unit, the Basal Sands and Gravels, consists of a complicated series of deposits containing matrixsupported, well rounded siliceous particles, frequently 100-150 mm in diameter. These pebbles are often crudely oriented in field exposures, suggesting en masse movement. Discrete lenses of generally matrix-poor, finer-grained material (coarse sands and fine gravels) are common throughout the deposits, both vertically and laterally, as are lenses of laminated clayey silts. Conditions of periodic streamflow, ponding and gentle slumping, as a result of mudflow processes, are indicated. SEM examination suggests that the silt and sand-sized material from all these deposits originates from a mixed provenance and an equally mixed range of environments of modification. The sands are probably derived from local tills and fluvial sediments, introduced into the cave after a greater or lesser mixing of the original sources.

The Intermediate Complex comprises a group of deposits that are lithologically transitional between the Basal Sands and Gravels below and the Breccias (infra) above. In general terms, the Complex may be characterized by the presence of siliceous pebbles, badly sorted coarse sand and highly altered limestone clasts. Internal stratigraphical relationships between different areas are unclear, due to the discontinuous nature of individual

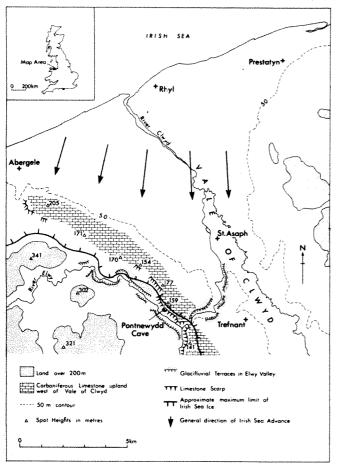


Fig. 1 Pontnewydd Cave. Location and geomorphological setting.

lenses and extreme lateral variability. Some occurrences show the combined effect of syngenetic growth of carbonate concretions and input of large quantities of organic matter. Microscopically, the grain textural suite combines qualities of both the underlying and overlying deposits, although the grain surface textural modification is basically fluvial in origin. Nowhere are primary fluvial deposits clearly evidenced; the matrix-supported fabric and poor sorting indicate that mass movement was again the last major emplacement process involved.

The two units of the Breccias comprise many angular coarse particles, mainly limestone, with medium to strong carbonate cement. Compared with the Lower Breccia, the Upper Breccia contains much more silt and fine sand, fewer but more severely fractured siliceous pebbles, and abundant but considerably less altered limestone clasts. The units are separated by stalagmite growth and laminated silts in some places. Under SEM, the Breccias are distinctly different from the Basal Sands and Gravels, although they show similar intra-sample variation. There are either provenance variations or process modification differences, or both, between the two Breccias. Whilst both contain mainly mechanically crushed quartz grains, surface modification, of only limited extent in both cases, is primarily fluvial in the Lower Breccia but both fluvial and diagenetic in the Upper Breccia. These deposits are the result of mudflow on a major scale, originating from the direction of the cave entrance. This process was interrupted once, during a period of relative quiescence, by limited fluvial (local drainage) and pool sedimentation, together with the growth of stalagmites, in what was probably a closed cave environment. The Breccias incorporate reworked material from the underlying deposits and, at one point, the Upper Breccia has clearly channelled the Lower Breccia and Intermediate Complex; this is consistent with the mode of emplacement envisaged. The limestone component and some of the silts and fine sand result from repeated inputs of entrance facies sediments. Alteration and cementation of the Breccias occurred before the entry of the next deposit.

There are no recognizable stable surfaces within the Breccias or the Intermediate Complex. Artefacts and fauna, which occur throughout the former and in certain deposits of the latter, would have been subject to transport and reworking like any other particle during mass movement.

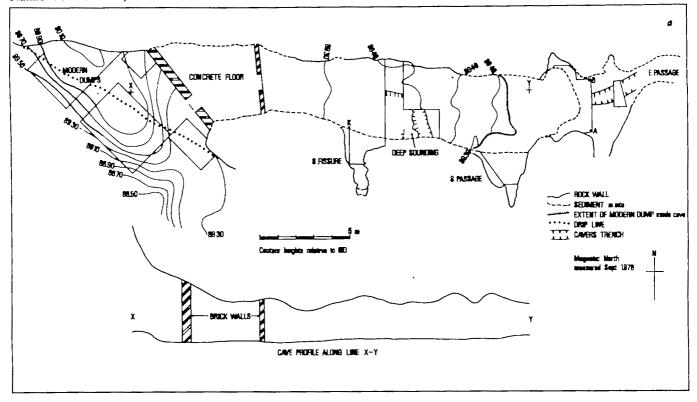
In the South Fissure only, the Breccias are overlain by a small remnant of uncemented, silty Red Cave—Earth, with abundant angular limestone clasts. These clasts lack all signs of chemical alteration but are slightly edge-rounded, indicating derivation from unweathered entrance facies sediments by mass movement. The quartz grains are texturally different from those of the Breccias but further analysis is necessary before any interpretation can be made.

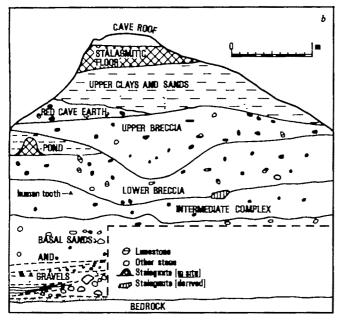
A series of current-bedded clays and sands is present in all areas, overlying either the Red Cave-Earth or the Breccias and is the result of an effluent stream that is clearing a choked drainage system. This unit (the upper clay and sands) is followed conformably by a thick stalagmitic floor.

Table 1 Comparison of measurements on the Pontnewydd molar and M<sup>2</sup> of other Pleistocene hominids last glaciation Neanderthal sample recomputed from data in ref. 33.

		H. erectus (Peking) <sup>17</sup>		European middle Pleistocene <sup>20,21</sup>			Krapina <sup>23</sup>			Last glaciation Neanderthal			Early Upper Palaeolithic <sup>33</sup>			
Measurement	Pontnewydd 1	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n
Length (mesio-distal)	10.9 (11.2 allowing for mesial wear)	10.9	0.7	7	11.9	0.3	3	11.3	0.4	14	10.6	0.7	12	10.6	0.8	9
Breadth (bucco-lingual)	12.9	12.7	0.5	7	13.5	0.4	3	12.8	0.2	14	12.9	1.2	12	12.1	0.7	9
Crude crown area (1×b)	140.6–144.5	138.6	8.0	7	160.6	5.3	3	144.3	7.2	14	136.8	17.0	12	126.8	15.9	8
					'Mode	n man	,17									
Root height	15.8 (lingual)	14.6 (m	1.6 nean)	7		5-13.6										
Root length	9.5	8.1	0.6	5												
$(\mathbf{m} - \mathbf{d})$	(preserved portion only)															
Root breadth (b-1)	12.3 (preserved) portion only)	12.2	0.6	5												
Root rubusticity (1×b)	116.9 (preserved portion only)	98.3	11.9	5		79										

Standard deviations (s.d.) and sample size (n) as indicated. Measurements in mm or mm<sup>2</sup>.

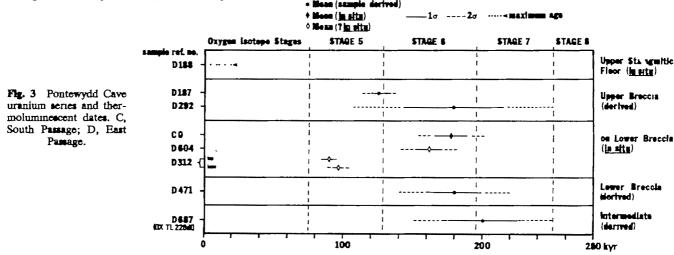




#### Fig. 2 Pontnewydd Cave. a, Plan; b, composite section

#### Chronology

(1) Uranium series dating-The principles involved in the dating of travertines from cave-sites are described elsewhere<sup>5</sup>. The stratigraphical contexts of the dated samples from the South and East Passages and their locations in the cave are given in Fig. 3. Four of the samples were probably in situ. Of these D188 yielded a maximum age of 20 kyr, suggesting a late Devensian or Holocene age for the stalagmitic floor which caps the sequence; CO and D604 are important as, in combination, they yield a minimum age of 170 kyr for the emplacement of the Lower Breccia in which the bulk of the archaeological finds is contained. The determination of 180 ± 20 kyr (D471), on derived stalagmite from the Lower Breccia, is consistent with this minimum age. Two dates centred on 90 kyr from a small stalagmitic boss probably in situ on the Lower Breccia (D312) suggest a long period of quiescence before the incoming of the Upper Breccia, and a further date of 125±6 kyr (D187), equivalent to Oxygen Isotope Stage 5e (ref. 6), on a stalactite incorporated into the Upper Breccia seems to confirm this and suggests that the solifluction of this layer occurred at a later cold stage. We believe that the cave had been sealed in the entrance area by the Lower Breccia deposit and so remained until this plug was breached during downcutting (through drift) of the



Elwy Valley<sup>7</sup>, resulting in the emplacement of the Upper Breccia deposit. This view is supported by oxygen isotope analysis of single growth layers of stalagmite D312 which show it to have been deposited in equilibrium with its feed waters, a characteristic of deposits found in sealed caves<sup>8</sup>.

(2) Uranium relative dating—Ten bones including one modern dog femur, seven bear bones representative of the different levels in the cave, and the human mandible and vertebra were submitted for relative dating. Post-depositional uptake of uranium series elements was assessed by counting  $\beta$ -particle emissions. All the bones, except the modern dog, gave counts higher than background, and it can be inferred that they, including the two human bones, are of some antiquity. The bones from the Upper and Lower Breccias, however, could not be distinguished, suggesting that they are not of greatly differing ages.

(3) Thermoluminescence dating-The ages obtained for emplacement of the Lower Breccia directly date neither the contained 'industry' nor the fauna beyond giving a minimum age of 177 kyr. We are fortunate in that a thermoluminescence (TL) date has been obtained from a globular burnt flint core from the Intermediate Complex immediately below the base of the Lower Breccia in the East Passage. The TL date is significant not only because it probably dates the use of fire on the site, almost certainly to be associated with the human occupation, but also because the hominid tooth described below was discovered within the same layer and in close proximity to it (Fig. 3). The date obtained is 200 ± 25 kyr (OXTL 226d1). The burnt flint core (D687) has been dated using the method previously described for flint10. TL parameters are as follows: Archaeological dose  $(29.6\pm1.0)$  krad for 1–8  $\mu m$  grains;  $(28.7\pm1.0)$  krad for 90–150  $\mu m$  grains; a value 10.085; plateau region 375– 475 °C; internal dose 0.025 rad yr<sup>-1</sup>. Soil  $\gamma$  + cosmic dose: 0.120 rad yr<sup>-1</sup> derived from fluorite dosimeter measurements in the layer. (Cosmic ray dose 0.003 rad yr<sup>-1</sup>, on site y spectrometer measurement.) Excavation water content 0.8 of saturation, which was 25% expressed as a percentage of dry weight. Assumed soil water content over the burial period  $(0.7 \pm 0.3)$  of saturation. Subsidiary measurements for Lower Breccia, Intermediate and immediately underlying Basal Sands and Gravels indicate radioactive homogeneity. TL age (200± 25) kyr: lab. ref. no. OXTL 226d1. The error quoted is the total predicted error at the 68% confidence level calculated as previously described12,13

#### **Hominid** finds

Of the three hominid finds, one, a tooth (Pontnewydd 1) whose stratigraphical position is indicated in Fig. 2b, is described below. The others, an immature mandible fragment (PN 2) and a vertebra (PN 3), are both from unstratified contexts but uranium relative dating indicates no difference in age between these specimens and the remainder of the Pleistocene fauna.

The human tooth from Pontnewydd is a left upper molar, probably an  $M^2$  of an adult individual. It has a large and low

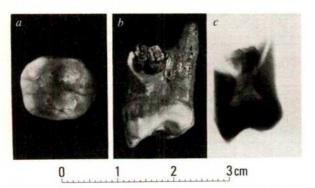


Fig. 4 The human molar: a, occlusal view (distal side at bottom); b, distal view; and c, radiograph of distal view.

25mm



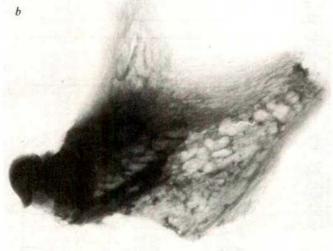
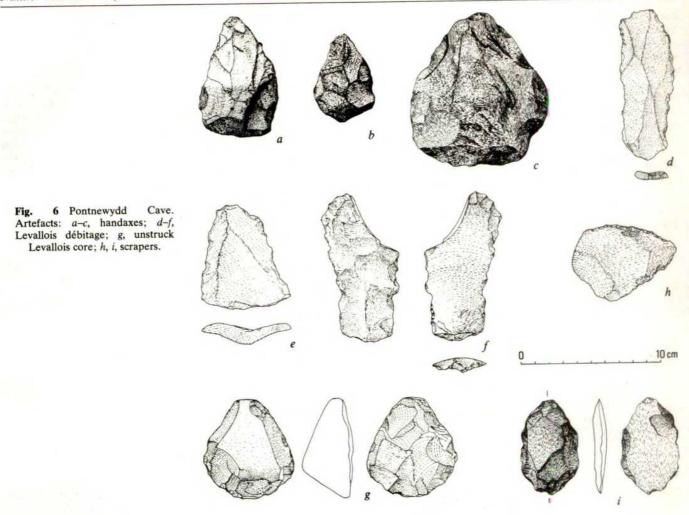


Fig. 5 The mandibular fragment: a, medial view; b, medial radiograph.

rectangular crown and a complete lingual root, but the buccal roots are broken (Fig. 4a, b).

The molar has four cusps. The protocone is the largest, the paracone is slightly larger and higher than the metacone, while the hypocone is small. The buccal cusps are higher than the lingual cusps and initial dentine exposure is apparent on the protocone. An oblique crest joins the protocone and metacone, and there are only moderately marked buccal and distal grooves. The lingual crown surface is more convex than the buccal surface, but there is no Carabelli's pit or cusp on the lingual surface, nor is a cingulum present. There are clear interstitial contact facets on both mesial and distal surfaces. The buccal roots are still fused at the point where they are broken, and taurodontism (enlargement of the pulp cavity) is associated with coalescence of the roots over half the length of the lingual root<sup>14</sup>. When intact the roots apparently barely diverged from each other, but they show a slight distal inclination.

Radiographs (Fig. 4c) of the molar show marked enlargement of the pulp cavity into the roots, which assume a prismatic shape. The form of the pulp cavity corresponds to Kallay's category of 'radicular endotaurodontism'<sup>15</sup>, where the cavity displays an hour-glass shape. This form of taurodontism is present in  $\sim 40\%$  of permanent molars examined from the last interglacial–early



last glaciation site of Krapina, Yugoslavia<sup>15,16</sup>. The Krapina sample displays the highest reported frequency and degree of such taurodontism, but taurodontism is not confined to Neanderthal fossils, nor is it characteristic of them in general. Comparative data on the distribution of this character are lacking for many middle Pleistocene hominids, although the Choukoutien and Mauer dentitions show a moderate degree of taurodontism<sup>17</sup>. On the other hand, the Bourgeois–Delaunay molars, stratified both in and immediately below a stalagmitic floor attributable to a temperature phase on pollen analytical grounds but dated by the U–Th method to  $146\pm16$  kyr, are reported to show none<sup>18,19</sup>. Therefore the Pontnewydd molar may represent one of the earliest known examples of this degree of taurodontism in the fossil hominid record.

Table 1 lists measurements for the Pontnewydd molar and a comparative set of measurements on the M2 of a Homo erectus sample, and of selected European middle and upper Pleistocene hominids. The crown dimensions and crude crown area of the Pontnewydd molar are close to the mean values of the Homo erectus and Krapina Neanderthal samples. The crown dimensions of the tooth also fall well within the size range of a last glaciation European Neanderthal sample, and within the upper limit of the range of an early Upper Palaeolithic sample. However, the molar is smaller than those of the middle Pleistocene sample (Petralona 1 (ref. 20), Arago 21 and the Bilzingsleben M<sup>1</sup> or M<sup>2</sup> (ref. 21)), while probably larger than that of the Steinheim specimen (comparative data on original not available). The Pontnewydd molar is identical in breadth to the mean value of a last glaciation Neanderthal sample, but is longer than most such specimens. Data on root dimensions are unavailable for many fossil hominids, but comparisons with Weidenreich's data (Table 1) suggest that the Pontnewydd molar has long and robust roots.

If the molar is an M<sup>2</sup>, then the complete root indicates an individual of at least adolescent age. Furthermore the presence of a distal interstitial wear facet suggests that M<sup>3</sup> was also in occlusion. However, the moderate degree of occlusal wear on the Pontnewydd molar suggests that this tooth derived from a young adult individual.

Some preliminary comments can be made about the immature mandibular fragment (PN2) and the vertebral fragment (PN3). The mandibular fragment represents part of the right ascending ramus, together with the crown of a molar, without developed roots (Fig. 5a). The ascending ramus lacks the upper parts of the coronoid and condylar processes and the lower border, and its minimum breadth is 31.5 mm. Exact identification of the molar is difficult, but it is small (mesio-distal length 11.2 mm; buccolingual breadth 10.5 mm; crude crown area 117.6 mm2) and has a simple five-cusped pattern. From the condition of the molar and the fact that radiography (Fig. 5b) shows the absence (presumed developmental) of a more posterior molar, three possible ages for the individual could be proposed, based on modern developmental patterns, assuming the preserved molar is an (1) M1, (2) M2, (3) M3, respectively. The first possibility, implying an age of <3 yr, can be excluded by the size of the ascending ramus. The second possibility, implying an age of 8-9 yr, would enable the size of the ascending ramus to be matched with those of earlier upper Pleistocene hominids such as Teshik-Tash 1 and Irhoud 3, which are at broadly comparable developmental stages22. In this case the relatively large ascending ramus would favour its identification as a non-anatomically modern specimen. The third possibility would imply a developmental age of >11 yr; the ascending ramus dimensions would then favour the identification of an anatomically modern, rather than archaic, morphology. However, whether the molar is an M2 or M3, its simple crown morphology, without the accessory cusps so often found in middle and earlier upper Pleistocene specimens, and its small size (near or below the minimum values for length and area in the large Krapina sample<sup>23</sup>) suggest caution about identifying the mandibular fragment as Middle Pleistocene in age.

The vertebral fragment represents the posterior and inferior parts of the body of a middle thoracic vertebra, probably from an adult individual. Two costal facets are preserved on the inferior part of the body, but the fragmentary nature of the specimen and its unremarkable morphology preclude further comment.

#### Pleistocene mammals

Faunal remains occur in three successive lithological units, the Intermediate Complex and the Lower and Upper Breccias. The following list includes the mammals from the Lower Breccia and Intermediate Complex of the East Passage only.

Ochotona pusilla: Pika Lepus cf. timidus: Hare Castor fiber: Beaver

Lemmus lemmus: European lemming Ar vicola terrestris: Water vole Microtus oeconomus: Northern vole Microtus gregalis: Tundra vole Apodemus sp.: Mouse Vulpes vulpes: Fox Ursus sp.: Bear Panthera leo: Lion

cf. Panthera sp.: Leopard-sized cat

Equus sp.: Horse

Dicerorhinus kirchbergensis: Rhinoceros

Cervus elaphus: Red deer Rangifer tarandus: Reindeer Bos or Bison sp.: A bovid Ovis cf. antiqua: Sheep

With the exception of Dicerorhinus kirchbergensis, widely regarded as an interglacial browser<sup>24</sup>, these animals could be grouped into a fairly unremarkable cold climate assemblage, although most of the larger species tolerate a wide range of thermal environments. Nothing in this collection indicates a date more precise than the later part of the Pleistocene. Sutcliffe and Kowalski<sup>25</sup> have suggested that Microtus gregalis is confined to the Devensian Stage (Last Glaciation) in Britain, even though it is recorded from earlier deposits in adjacent parts of continental Europe. This proposal would seem unlikely. The characteristic rodent assemblage of the colder regions of the northern continents was well established in the early Pleistocene<sup>26</sup>, and small mammal faunas closely parallel to that of Pontnewydd still exist over wide areas of northern Eurasia, Alaska and Arctic Canada. Successive cold stages during the Pleistocene could have led to the spread of rather similar faunas into Britain, particularly as low sea levels would allow free migration from the continental landmass. One would not expect any one form to be specifically excluded from the British region under these circumstances and the occurrence of Microtus gregalis from a pre-Devensian context is not surprising. Note here the first record of Ovis cf. antiqua in Britain.

The fragmented and abraded nature of the animal remains, together with the occurrence of robust limb bones that have been shattered and bent into bizarre shapes, support the suggestion that the Breccias have been sludged into their present position. Any original stratigraphy seems to have been completely destroyed and the fauna almost certainly has been mixed by this sludging process, but the abundance of carnivore remains, particularly those of adult and juvenile bears, implies that a genuine cave assemblage is present.

We make no comment about the occurrence of animals, noted by Boyd Dawkins<sup>2</sup> and McKenny-Hughes<sup>3</sup>, that would now be assigned to the Ipswichian s.s. ('Last Interglacial'). These include *Hippopotamus amphibius*, *Palaeoloxodon antiquus*, *Dicerorhinus hemitoechus* and *Crocuta crocuta*. None of these records have been substantiated by recent excavation. The evidence of stable isotope analysis that the cave may have been closed during the Ipswichian leaves open the possibility that the animals may have come from a localized entrance deposit, since destroyed.

#### Archaeological material

The new archaeological finds (Fig. 5) comprise some 300 artefacts. Stratified examples were located, along with the fauna, within the breccia units and in the Intermediate Complex. No in situ settlement survives and all of the artefacts have been transported into the cave by mass movement. We cannot demonstrate that the finds represent a single industry but the location of the site far outside the known distribution of British Acheulian sites would render multiple occupation less likely. It is uncertain as to what part of the fauna may constitute the food bones of the Palaeolithic hunter-gatherer community (or communities).

Combination of the TL and uranium dates and faunal evidence suggests that the occupation(s) took place within the time range of Oxygen Isotope Stage 7/earlier Stage 6. This dating makes sense in climatic terms in so far as the occupation seems to have preceded the onset of glaciation later in Stage 6 when deep-sea core data<sup>27</sup> indicate an increase in ice-volume, and stable isotope studies of northern England speleothems<sup>28</sup> suggest glaciation from 170 to 140 kyr. Radiometric dating of the Barbados coral terraces<sup>29</sup> suggests a major eustatic lowering of sea level, bracketed by the Kendal Hill and Rendezvous Hill terraces between 180 and 125 kyr.

The archaeological material has been considered elsewhere<sup>30</sup>. The principal artefactual components are handaxes of Acheulian types, other implements (principally scrapers) and Levallois débitage (including cores) (Fig. 6). The nature of the artefacts and small size of the potential living-area in the cave entrance suggest that the cave was probably used as a temporary butchering site. The industry finds little close comparison with other sites either in Britain or in adjacent continental areas.

Of the 300 artefacts recovered about 40 are of flint or chert and most of the remainder are of siliceous igneous, pyroclastic and volcaniclastic rocks. These specimens, so far only examined macroscopically, show evidence of having suffered a low-grade of metamorphic recrystallization, whilst in addition a cleavage is variably developed within the rocks, being most pronounced in the fine-grained specimens.

The variety of rock types, the low grade metamorphic crystal-lization and the poorly developed cleavage, suggest a source either in North Wales or the English Lake District. Their presence on the site, whether as artefacts in the Breccias or as unmodified pebbles in both the Breccias and Basal Sands and Gravels, suggests glacial transport to the Elwy Valley before oxygen isotope Stage 6. Whatever the origins of the other rocks, the flint (which has been recognized so far only from the Breccias) must have come as a component of the Irish Sea drift<sup>31</sup>. This drift may be compared with the "Main Irish Sea Glaciation", of Embleton<sup>32</sup> whose hypothesis of the cutting of the lower Elwy Valley as a result of local glacial diversion of the river by inland penetration of Irish Sea Ice would then presuppose another such event earlier in the glacial Pleistocene, unrecognized as yet in the local terrestrial till sequences.

The Pontnewydd excavations will continue for several more seasons to increase the statistical validity of the artefactual and other samples; to improve the dating and comprehension of the stratigraphy; to establish the relationship of the site to the local Quaternary geology and geomorphology; and, if possible, to recover more hominid remains.

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Williams for photographic reduction and mounting of the figures and Monica Cox for typing the manuscript. The site being a scheduled ancient monument, the work proceeded with the sanction of the Ancient Monuments Branch of the Welsh Office,

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and its principal inspector, Dr M. W. Thompson. The finds are lodged in the National Museum of Wales by gift of the landowner, Major David Williams-Wynn whose interest and support has made these discoveries possible.

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# Intragenic amplification and divergence in the mouse $\alpha$ -fetoprotein gene

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The DNA sequences of the 14 exon junctions in the murine  $\alpha$ -fetoprotein gene were determined using cloned genomic DNA. When these exons were examined with respect to the polypeptide segments they encoded, a direct correspondence between a threefold repeat of four exons and three protein domains was observed. Nucleotide sequence comparisons among the four exons of each domain were used to deduce the likely structure of the primordial domain, and the order and mechanism of its triplication to form the tripartite ancestral gene from which both \alpha-fetoprotein and serum albumin arose. Sequence homologies among the four exons that constitute a single domain also suggest that they were derived, at least in part, from a common sequence which underwent successive amplification and divergence.

MAMMALIAN α-fetoprotein (AFP) and serum albumin are useful models for elucidating early events in the evolution of a multi-gene family. Their genes arose through duplication of an ancestral gene 300-500 Myr ago<sup>1-6</sup>. They have remained linked on chromosome 5 in the mouse, with the albumin gene 14 kilobases (kb) of DNA upstream from the AFP gene

Brown<sup>9,10</sup> first suggested that the ancestral gene itself had been formed by amplification and divergence of a simpler sequence when he recognized a thrice-repeated pattern of cysteine-cysteine disulphide bridges in the amino acid sequences of human and bovine albumins. He concluded that the albumin gene must have arisen from the triplication of a primordial domain, consisting of ~190 amino acids. The amino acid sequence of murine AFP also shows this threefold repeat pattern1,6

We have inquired into the genetic basis for the tripartite structure of the AFP/albumin ancestor. An examination of the structures of their genes in the mouse<sup>2</sup> revealed that both are comprised of 15 coding blocks or exons, which are interrupted by 14 intervening sequences. The sizes of the 15 corresponding exons in each gene were estimated by electron microscopy of R-loops, and shown to be very similar. Of particular interest was the observation that the 12 internal exons of either gene, numbers 3-14, seemed to be composed of three similar sets of four exons, based on their sizes. We therefore proposed that the three protein domains had been generated by the triplication of a primordial gene consisting of four exons<sup>2</sup>. To test this, the borders of the 15 exons of the AFP gene were located by DNA sequencing using cloned genomic DNA.

#### Nucleotide sequences of the splicing iunctions of the AFP gene

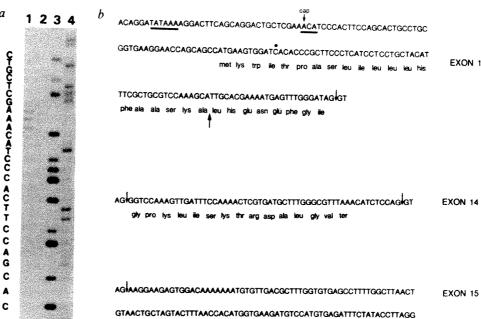
The borders of the exons in the mouse AFP gene (see Fig. 1) were determined by the procedure of Maxam and Gilbert 17. In some instances where the determination of a splicing site was unequivocal from sequences of one side of the intron, the sequence at the other side was not determined. The intron junction sequences closely resemble the consensus sequence suggested by Lerner et al. 12, and no exceptions to the GT-AG rule proposed by Breathnach et al. were seen 13. Indeed, because of the presence of sequence redundancy at 12 of 14 borders, the exact locations of the splice sites can only be assigned on the basis of these four invariant nucleotides.

The location of the mRNA cap site was determined by an S<sub>1</sub> nuclease protection experiment<sup>14</sup> shown in Fig. 2a. A 100nucleotide DNA fragment, labelled on the anticoding strand at a Sau3A site in exon 1, was hybridized to AFP mRNA. The S1 nuclease-resistant fragments (lane 1) were sized on an acrylamide gel by comparison with chemical degradation products of the same DNA fragment (lanes 3, 4). Three prominent bands, 42-44 nucleotides upstream from the AUG initiation codon, were observed. From a comparison of the nucleotide sequence at the putative cap site with previously reported cap site sequences in other genes<sup>15,16</sup>, we have tentatively identified the first adenine residue of the underlined ACA triplet in Fig. 2b as the cap site. The other bands presumably reflect imprecise cutting by S<sub>1</sub> nuclease as they did not decrease with increasing S<sub>2</sub> nuclease concentration (lane 2). A 'TATAA' sequence, or

		Exon #	Exon Size (BP)	3' Exon Junction	5'	IVS	3'	PANCLION 2, Exon	Exon
		1.	129	GGG ATA G		GIAAGAT ··· TTTTCAG	1	CT TCC ACG	2.
		2.	52	CTT AGC AT		GIAAGTT ··· CTTGCAG		A SCT ACC	3.
Γ	A	3.	121	GAA AGC CAG		GIGAGTG ···		CTA TCT 6T6	4.
	8	4.	212	ATG AAC AG		GIAAGGA ···		6 TTC ATC	5.
1	C	5-	133	CAG ACA AAG		··· CATCTAG		AGA GCA TCC	6.
L	Đ	6.	98	CAG SCA AC		GIAAGTA ···		A ACC ATT	7.
Γ	A	7.	130	CAG GAT GGG		GIAGGAG ···		GAA AAA GTC	8.
1	B	8-	215	ATG GCA AG		··· ATTTA <u>AG</u>		C 111 CTT	9.
11	¢	9.	133	GAC AAT CTG		GIAGGTT ···		GAA GAA GAA	10-
L	B	10-	98	CAA AAT CT		··· CTTCAAG		6 TTC CTT	11.
Γ	Ā	11.	139	SAS SGA ATS		GIGAGTS TTAACAG		GCC GAC ATT	12.
	8	12.	224	AAA CAA GA		··· ACCCCAG		6 CTT CTC	13.
111	C	13-	133	ACA GAA GAG		GIGCETA ···		GGT CCA AAG	14.
L	D	14.	55	TCTCCAG		GIAAGAA ··· ATTGCAG		AAGGAAGA	15.
		15.	141						
			C	ONSENSUS: AG	i	GIAAGT\$ ··· TTCCAG			

Fig. 1 The sequences at the splice junctions in the AFP gene. The partial DNA sequences spanning the 14 splice junctions of the murine AFP gene were determined using cloned genomic fragments<sup>2</sup> and the procedures of Maxam and Gilbert<sup>11</sup>. The tentative locations of the 15 exons were originally determined by R-loop techniques and a direct comparison of a detailed endonuclease restriction map of the cloned genomic fragments with the restriction map of its cDNA <sup>1,2,46</sup>. The positions of the junctions, indicated by the vertical lines and the sizes of the 15 exons, shown in the first column, were deduced by comparing the genomic and cDNA sequences <sup>1</sup> and by the presence of the invariant GT and AG dinucleotides (underlined) at the 5'- and 3'-intervening sequence borders, respectively. The sequences of the last two or three complete in-frame exon triplet codons at the 3' junction are followed in each instance by the first seven nucleotides of the adjacent intervening sequence. The last seven nucleotides of that intervening sequence and the first two or three complete triplet codons of the next exon follow the dotted line which represents those remaining intervening sequences. The in-frame triplet codons are designated by the spacing of the exon sequences. The consensus sequence of Lerner et al.<sup>12</sup> for the splice junctions is shown at the bottom. The designations used in the text for domains (I, II and III) and subdomains (A-D) in the gene, are shown on the

Fig. 2 The sequence at the 5' and 3' termini of the AFP gene. a, The approximate location of the first nucleotide of AFP mRNA was determined by an S<sub>1</sub> protection experiment<sup>14</sup>. One microgramme of fetal yolk sac poly(A)\* mRNA, which is 10-20% AFP mRNA, was hybridized to a denatured 100-bp Sau3A-HincII fragment derived from pAFP14Z (ref. 2), which was end-labelled at the Sau3A site within exon 1 on the anticoding strand (asterisk in b, excn 1). The hybridization and S, nuclease digestion were performed according to the procedures of Favaloro et al. 47. The pro-tected DNA fragment was electrophoresed in a 10% urea acrylamide gel. Lanes 1, 2: S<sub>1</sub> nuclease (Miles) used at 200 and 500 U ml<sup>-1</sup>, respectively. Lanes 3, 4: To size the S<sub>1</sub> digest gel migration, the same Sau3A-HincII fragment was subjected to chemical degradation<sup>11</sup> for the G reaction (lane 3) and A reaction (lane 4). The complement of the determined sequence is shown on the left, so as to correspond to the coding strand. The sizes of the S<sub>1</sub>-protected fragments were determined by comparison with the nucleotide sequence, taking into account the fact that the chemically degraded fragments lack the modified base. b. The nucleotide and amino acid sequences of the first and last two exons. In exon 1, the



<u>AATAAA</u>AACTTTTCAACTATTTCTCTTCTCCTAGTCTGCTTTTTTTAT

underlined ACA trinucleotide identifies the likely position of the cap sequence, as determined in a. The underlined TATAA sequence 30 bp upstream from the cap site is the Hogness box. The 3'-splice junction is shown by a downward arrow and the junction between the 20-amino acid signal peptide and the mature protein is designated by the upward arrow. The Sau3A site labelled in a is shown by the asterisk. In exon 14, the splice junctions are designated by the downward arrows and the terminator codon TAA is shown as 'ter'. In exon 15, the 5'-splice junction is shown by the downward arrow. The signal for poly(A) addition, AATAAA, is underlined and the upward arrow designates the poly(A) addition site<sup>6</sup>.

Hogness box, thought to be required for precise initiation by RNA polymerase II, is found 30 base pairs (bp) upstream from the cap site (Fig. 2b).

The termination codon for AFP synthesis, TAA, occurs in exon 14, as shown in Fig. 2b. The 3'-untranslated sequence in this exon differs at three positions from our previous report¹. Although we cannot eliminate sequencing errors as an explanation, it is likely that these result from the fact that the cDNA and genomic clones were constructed from different inbred strains. The sequence in Fig. 2 agrees with that of Law and Dugaiczyk⁶. Exon 15, the last exon of the AFP gene, contains only 3'-untranslated sequence. The poly(A) addition signal, AATAAA¹7.18, is 120 bp from the start of this exon (Fig. 2b) and the poly(A) addition site is an additional 15 bp downstream⁶.

# Coincidence of genetic and functional domains

An examination of the sizes of the internal 12 exons in the AFP gene (Fig. 1) strengthens the original premise of a thrice-repeated pattern. The gene can be readily divided into three sets of four exons designated subdomains A-D based on their sizes; this excludes the first two and the last exons. Subdomains vary in size within families by multiples of 3 bp, and thus any insertions or deletions within subdomains do not change the reading frame of downstream exons. There is also conservation of the location within a triplet codon of the 5' and 3' borders of each family (Fig. 1).

Figure 3 shows the pattern that emerges when the borders delineating the exons are superimposed on the protein. It is immediately apparent that all the exons of any one family encode segments of the protein that are identical with respect to the location of the disulphide bridges. For example, each subdomain C (closed circles, Fig. 3) encodes an equivalent double loop region or subdomain in each of the three domains.

Are the three genetic domains defined by the four-exon repeat coincident with functional domains in either AFP or albumin? One can consider the first and last exons, which lie outside the domains, as functionally distinct in that the first exon encodes the signal peptide and the last exon encodes the

3'-untranslated sequence and poly(A) addition signal. In many but not all secreted proteins, the signal peptide exon is physically separated from the rest of the gene.

The active binding sites in mammalian AFPs are poorly characterized. In the case of albumin, experiments on the intact protein and on isolated fragments corresponding to domains have localized binding sites to specific domains. The high-affinity long-chain fatty acid binding site is in domain III, the bilirubin binding site in domain II and the indole binding sites in domain I Domains I and II exhibit weaker fatty acid binding sites, suggesting that these are remnants of the high-affinity site in domain III, the putative primordial domain (see below). Interestingly, copper(II) or nickel(II) binding is performed by the first three amino acids of the mature protein, which is separately encoded by exon 1<sup>19</sup>. These studies are consistent with the notion that the protein domains are functionally distinct and that the selective pressure to maintain amplified domains lay in the generation of functional diversity within the protein.

The relationship between genetic and functional domains has been clearly demonstrated only in instances where there has been intragenic amplification and divergence, as has occurred in the AFP/albumin ancestral gene. The other compelling example occurs in the immunoglobulin heavy-chain constant region genes, where each of the four constant region gene domains has been correlated with structural or functional protein domains<sup>20-25</sup>. Stein et al.<sup>26</sup> have reported a triplication of two internal exons in the chicken ovomucoid gene which possibly correspond to the three binding sites for protease inhibitor in the protein.

Amplification of genetic domains is facilitated by the presence of pre-existing intervening sequences; thus, as suggested by Gilbert<sup>27</sup> and Darnell<sup>28</sup>, intervening sequences serve to increase the probability of useful recombination during unequal crossing-over. Following amplification, the presence of intervening sequences then decreases the likelihood of genetic recombination and subsequent deletion of DNA, because the intervening sequences themselves rapidly diverge<sup>29</sup>. In the case of the albumin and AFP genes in the mouse, which are located<sup>8</sup>

Т	able 1 Sequence ho	mologies among subd	omains					
	z, Sequence homology	y within subdomain fa	milies					
	I vs II II vs III I vs II							
Α	36.4%	41.5%	34.7%					
	$1.2 \times 10^{-2}$	$6.8 \times 10^{-6}$	$6.9 \times 10^{-3}$					
В	38.8%	35.8%	32.6%					
	$2.7 \times 10^{-6}$	$1.3 \times 10^{-4}$	$5.9 \times 10^{-3}$					
С	46.6%	36.1%	43.6%					
	$2.9 \times 10^{-7}$	$1.7 \times 10^{-3}$	3.7×10 <sup>7</sup>					
D	32.7%	NS	NS					
	$4.0 \times 10^{-2}$							
Total	39.1%	36.9%	35.4%					
	$1.3 \times 10^{-12}$	$1.5 \times 10^{-10}$	$2.5 \times 10^{-1}$					
ь,	Sequence homology	between subdomains.	A and C					
	IC	IIC	IIIC					
IA	NS	32.2%	44.6%					
		$3.4 \times 10^{-2}$	3.7×10°					
IIA	40.0%	39.2%	40.8%					
	$3.9 \times 10^{-5}$	$1.1 \times 10^{-4}$	$1.7 \times 10^{-1}$					
IIIA	40.6%	41.4%	46.6%					
	$1.7 \times 10^{-5}$	$6.8 \times 10^{-6}$	2.9×10					

a, Nucleotide homologies of pairwise comparisons within subdomain families for the alignments illustrated in Fig. 4a are shown. In each case, the upper value represents the per cent homology, codon gaps not being taken into consideration. The bottom value is the probability (P) of finding this homology or more by chance, taking the length of the sequences being compared into account. It is based on the normal approximation to the binomial distribution, using the formula

$$p = 1 - \Phi\left(\frac{H - np}{\sqrt{npq}}\right)$$

where n is the standard length of the sequence, H is the number of homologous bases, p is the probability of finding a match at any position (in this case p = 0.25), and q = 1 - p. (NS = not significant.) b, Nucleotide homologies of pairwise comparisons between subdomains A and C are shown, using the alignments illustrated in Fig. 4a.

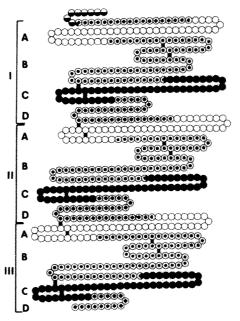


Fig. 3 The coincidence of genetic and protein domains. The 585 circles in the diagram represent the amino acids of mature AFP, drawn to connect adjacent cysteine-cysteine disulphide bridges, shown by the connecting bars<sup>1,10</sup>. The amino acids encoded by exon 1 ⊕, the three A subdomains ⊙, the three B subdomains ⊙, the three C subdomains ⊕ and the three D subdomains and exon 2 ♠, are drawn, and designated on the left of the diagram according to usage in Fig. 1 and the text. In cases where a splice junction interrupts an in-frame triplet codon, the amino acid is drawn with two symbols.

13.5 kb apart on chromosome 5, this has probably contributed to the maintenance of the two genes in tandem.

When one examines genes such as insulin<sup>30,31</sup>, lysozyme<sup>32</sup> and alcohol dehydrogenase<sup>33</sup>, in which there is no apparent intragenic amplification, the coincidence of genetic and functional domains is far less evident. Gilbert<sup>27</sup> has proposed that the selective advantage of intervening sequences lay in the ability of a cell to recruit by translocation and recombination discrete genetic domains for the assembly of new genes, thereby markedly increasing the rate of evolution. One test of such a model would be the demonstration that genes are composed of genetic elements or exons encoding specific functions of the protein. Structural data, and the observation that the haem binding site of  $\alpha$  or  $\beta$  globin is encoded by the middle exon of the three-exon gene, have been cited by several groups as support for this model  $^{31,32,34-36}$ . Particularly intriguing is the finding that this exon is in fact split in leghaemoglobin of soybean  $^{37}$ , as was predicted by  $^{36}$  on the basis of structural arguments alone.

#### Mechanisms of divergence between domains

A comparison of related subdomains provides an opportunity for identifying evolutionary mechanisms that have generated domain diversity. To facilitate such a comparison, each subdomain family was aligned using the optimal nucleotide sequence homologies found by the computer programs of Staden<sup>38</sup> and an adaptation of the Queen and Korn<sup>39</sup> program. The minimum number of in-frame gaps have been introduced to maintain lengths of uninterrupted homology.

Figure 4a shows the alignments for each subdomain family, and Table 1 enumerates the nucleotide homologies within and between subdomain families, together with the probabilities that the number of matches could have arisen by chance. The three C subdomains are identical in size, and appear to have incurred no insertions or deletions. They have been aligned in Fig. 4a with a two-codon gap to facilitate their comparison with the A family, discussed below. They are also the most closely homologous family, with a 47% nucleotide homology between IC and IIC, a 44% match between IC and IIIC and a 36% match

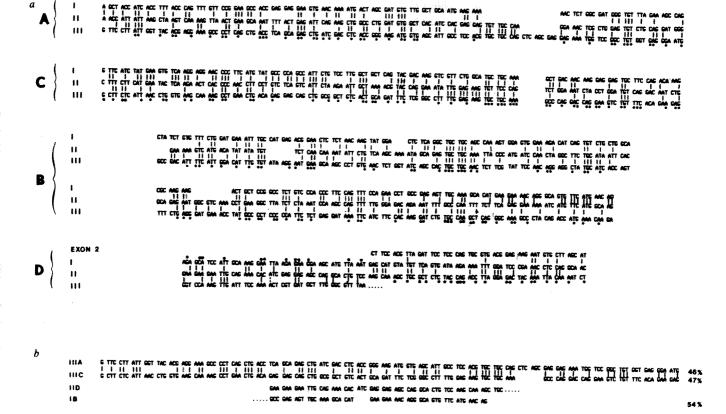


Fig. 4 The alignment of sequence homologies within subdomain families. a, The nucleotide sequences of each of the 13 internal exons are aligned in their corresponding subdomain families. Their alignments were determined by optimizing nucleotide sequence homologies in pairwise sequence comparisons within families. Within each family, homologies between domains I and II and domains II and III are indicated by the vertical lines, and the homologies between domains I and III are shown as asterisks below domain III. The sequences are displayed with spaces between in-frame codons, so that codon gaps in subdomains are evident. The two-codon gap in the C family alignment facilitates the comparison of the A and C families. b, The bottom alignment shows a comparison between representatives of each subdomain family, IIIA, IIIC, IID and IB. The entirety of IIIA and IIIC are aligned, and the vertical lines between them identify homologous nucleotides. The first 57 bp of IID are compared with internal regions of IIIA, IIIC and the last 50 bp of IB. The homologies between IID and each of these are shown on the right. The probabilities that these could have arisen by chance, using the formula in Table 1, are  $1.64 \times 10^{-4}$  (IID vs IIIA),  $5 \times 10^{-5}$  (IID vs IIIC) and  $1.3 \times 10^{-6}$  (IID vs III).

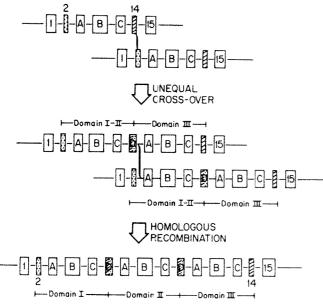


Fig. 5 A model to explain the triplication of the primordial domain. A model for the generation of the three domains in the ancestral AFP/albumin gene is illustrated. The seven exons comprising the entire primordial gene consisted of the present exon 1 containing the signal sequence, exon 2, subdomains A, B and C, exon 14 containing the termination codon, and exon 15. An unequal cross-over event between exons 2 and 14 resulted in the formation of a D exon. This created a two-domain gene, equivalent to domains I-II and III. A subsequent homologous recombination event then gave rise to domains I and II, as the result of a duplication of domain I-II. This model takes into account the sequence relatedness of the three domains in Table 1, as well as the chimaeric nature of the D subfamily.

between IIC and IIIC. The A subdomains each differ in size, and their homologies range from 35 to 42%, the longest, IIIA, being 139 bp long, 9 bp longer than IIA. A single gap in IIA, 30 bp from the 3' border, represents the most likely position of either a deletion in IIA or an insertion in IIIA. Subdomain IA contains an additional gap of three codons, which has been positioned to the 5' side of the gap in IIA. In this case, however, there are several other possible locations, and the alignment of the four codons to the right of the gap is tentative.

By comparing the A subdomains with their counterparts in the rat albumin gene, whose sequences were determined by Sargent et al. 40.41, it is possible to date approximately when these gaps occurred. Although subdomains IIA and IIIA are identical in size in both genes, albumin IA is four amino acids longer than AFP IA. Therefore, one would infer that the size difference between IIA and IIIA existed before the AFP/albumin gene duplication, tentatively placed at 300-500 Myr ago<sup>2</sup>, whereas the alteration in IA occurred after it.

Alignment of the B subdomain family requires that two members contain deletions, at different places in the sequence. As illustrated in Fig. 4, IB (212 nucleotides) contains two gaps, one of 9 bp and one of 3 bp. IIB (215 bp) contains a gap of one codon at its 5' border, which is the only possible example of a mutation at a splice junction, and a second codon gap elsewhere. The alterations in the B subdomains are apparently unrelated, and probably arose independently after the domain triplication by deletions, for otherwise one must postulate two insertions at each of the gaps. This is in keeping with the finding by de Jong and Ryden<sup>42</sup> that deletions are far more common than insertions as evolutionary mechanisms of divergence. The members of the B family are less homologous to one another than are those of the A or C families, with pairwise homologies of 32–39%.

The D family of subdomains consists of four exons, exons 6 (ID), 10 (IID), 14 (IIID) and 2. This classification is based on several different lines of argument, as the total percentage homology between members of this family is less than in any of the other families (see Table 1). Exons ID and IID are identical in size, with the corresponding cysteine residue occurring at the same position, but only share marginal nucleotide homology. This is not surprising, given that of all the exons in the AFP gene, that encoding ID is the least homologous to the corresponding exon in the rat albumin gene<sup>41</sup>. Thus, we consider that there is little selective pressure to retain the constancy of the nucleotide sequence of ID, although there is obviously considerable pressure to maintain the size of this subdomain at 98 bp. The relationship between IID and IIID is most evident when each is compared with an internal region of either IIIA or IIIC. As shown for IID in Fig. 4b, its first 57 bp are 46 and 47% homologous to a 57-bp internal segment of IIIA and IIIC, respectively. Similarly, IIID is 45% homologous to the same region in either IIIA or IIIC (data not shown). Finally, the inclusion of the second exon in this family, which had previously been considered outside the domain repeats, is based on a 40% homology between it and the final 52 bp of IID, as shown by the asterisks below IID in Fig. 4a.

# Internal homologies shared among subdomain families

An examination of the evolution of the primordial domain itself was initiated when marked similarities between the A and C subdomain families became apparent. They are very similar in size and in the pattern by which the exon junctions occur in the reading frame. In fact, it is possible to align them as a sixmember family (see Fig. 4a), based on significant nucleotide homologies which they share (Table 1). As shown in Fig. 4b and Table 1, subdomains IIIA and IIIC are as homologous to each other, 47%, as the most homologous pair within either family, IC and IIC. Thus, subdomains A and C must have arisen from a duplication which preceded the formation of the primordial domain.

Several conclusions about the history of the A/C family can be drawn by comparing the six related subdomains. It is likely that the original A/C exon was 133 bp long, and that the additional 6 bp in IIIA resulted from an insertion (Fig. 4b). Otherwise at least two exons would have had to undergo independent but identically positioned deletions before their further amplification. For the same reason, the gaps present in IA and IIA are the likely consequence of deletions. It has been proposed, based on sequence analysis of globin genes in mammals, that deletions and insertions occur preferentially in regions composed of short direct repeats<sup>15</sup>. Evidence of such an event is seen when the 3'-untranslated sequence of the mouse albumin gene is compared with that of the rat<sup>8</sup>. If such a sequence was present in the primordial A/C subdomain, this could have allowed independent deletions at the same location. However, no such repeated sequence in IIIA is apparent today.

We next investigated whether sequences existed in the other two subdomain families, B and D, which would suggest a common ancestry with A and C. The results indicate that all four subdomain families exhibit regions of homology with one another. In particular, as aligned in Fig. 4b, the first 57 bp of subdomain IID are 54% homologous with the last 50 bp of IB. This same region of IID is homologous with the middle of IIIA and IIIC. A more detailed analysis of these inter-subdomain relationships is now under way, but it is already evident that all four exons that constitute a domain are related, and were probably initially derived, at least in part, from one exon.

The sequence of events leading from a single exon to the primordial domain, composed of exons of quite different sizes, must have included amplification of that exon (of as yet undetermined length) creating multiple adjacent copies separated by intervening sequences. Following their divergence, separate exons could have been consolidated by unequal cross-

overs during recombination or by removal of intervening sequences, similar to that which has occurred in a rat insuling gene  $^{30}$  and in a pseudo  $\alpha$ -globin gene in the mouse  $^{43}$ . Such a sequence of events has been postulated for the type  $1\alpha 2$  collagen gene of the chick, which contains multiple homologous 54-bp exons  $^{44}$ . Mutations within junction sequences could also result in exon border changes, altering the size of individual exons and adding segments unrelated in sequence to the initial exon. Such processes would eventually have led to the establishment of a primordial gene, the exons of which varied in size, but which were at least partially related in sequence. Brown  $^{9.10}$  and McLachlan and Walker  $^{45}$  have presented a

model to explain the generation of the primordial domain, based on the positions of the cysteine-cysteine bridges within a domain. Their model proposed that a 73-amino acid segment triplicated, and then a middle segment was consolidated by deletion. In genetic terms, their 73-amino acid segment would have been encoded by exons 2, A and 14, and the triplication would have generated a gene composed of 9 exons flanked by exons 1 and 15. The subsequent generation of exon B from the internal exons 14, 2, A, 14 and 2 must have involved deletions of both coding sequence and intervening sequences, leaving a primordial domain composed of exons 2, A, B, A (C) and 14. The homologies we observe between exon families certainly support a mechanism of this kind although the precise details of the deletions have not been elucidated. Note that the homologies between exons A and 14 also argue that intragenic amplification must have occurred before the establishment of the 73-amino acid segment.

#### Order of domain triplication

Pairwise comparisons between the nucleotide sequences of the three domains can potentially be used to trace the order of the two successive duplications of the primordial domain. The sequence homologies between domains reflect, in part, the evolutionary time between successive domain duplications, the most homologous corresponding to the most recent duplication. However, it should be emphasized that other evolutionary pressures exerted at both the nucleotide and amino acid levels have contributed to the rate of divergence of the three domains, and may serve to obscure the divergence based on time.

Although comparison of the homologies between entire domains (Table 1) shows domains I and II (39%) to be more closely related than domains I and III (35%), this difference is not statistically significant by a  $\chi^2$  test. Using this test, the only significant difference between the homologies within any subdomain family occurs in the C family, where the relatedness of domains I and II is significantly greater than that of II and III. Nevertheless, it is also evident that in three of the four families (B, C and D), domains I and II are the most homologous, and in subdomains A, B and D domains I and III are most distantly related. It seems likely, therefore, that domain II is the evolutionary link between domains I and III, and the order of triplication was domain III giving rise to a domain which subsequently duplicated to form domains I and II. A brief examination of rat albumin sequence40 reveals that the overall order of relatedness between domains is the same as that in AFP.

This order, which was originally predicted by Brown<sup>10</sup> based solely on amino acid homologies in bovine and human albumins, is supported by a model to explain the generation of the D subfamily. As discussed earlier, and represented in Fig. 4, this family is composed of two 98-bp exons, ID and IID, which are homologous at their 5' termini to exon 14 (IIID), and at their 3' termini to exon 2. Possibly, at one time exons 2 and 14 were each entire subdomain Ds, and in two independent events the beginning of exon 2 and the end of exon 14 were deleted. Alternatively, one could postulate a copy of D split, creating two separate exons. If an exon division did occur, it is difficult to envision how exons 2 and 14 came to lie on opposite ends of the gene.

We favour a model in which exons 2 and 14 flanked the primordial domain, corresponding to the A, B and C subdomains of domain III (Fig. 5). During the first unequal crossover in the triplication, these flanking exons fused to form subdomain D. This could occur in one step, with the cross-over occurring within the two exons, eliminating the termination codon and generating a two-domain protein. Alternatively, it could occur in two steps, the first step being a cross-over in the intervening sequences, followed by a fusion of exons 14 and 2. In the latter case, however, the resulting protein would still only comprise a single domain, until subsequent events removed the termination codon in the fused subdomain. This second alternative seems unlikely, as it would place the downstream domain under no selective pressure. In either case, the one strong constraint placed on the fusion would be that the reading frame of the downstream exons be preserved. The subsequent duplication of the left domain, by homologous unequal crossing-over, would generate domains I and II, in agreement with the overall relatedness of domains I and II over domain III.

The model in Fig. 5 differs slightly from our original proposal<sup>2</sup>

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in that the primordial genetic domain is composed of five rather than four exons, and exon D is only generated by the fusion of two of these during or after the first duplication. Thus, one could define a domain as consisting of the equivalents of exons 2, A, B, C and the first half of exon D. This is precisely the way in which the three protein domains in albumin were originally delineated 10.45. The second duplication of exons I-IIA, B, C and D is in keeping with our original model.

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# Membrane asymmetry in epithelia: is the tight junction a barrier to diffusion in the plasma membrane?

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Membrane-bound lectins and some lipid probes are incapable of passing through the tight junction region of epithelial cell membranes. The lectins are immobile on the cell surface, but lipid probes diffuse freely in the membrane. The ability of a lipid probe to pass the tight junction is correlated with its ability to 'flip-flop' to the inner monolayer of the cell membrane bilayer.

EPITHELIA form tight monolayers of cells in many organs and control the passage of solutes across the monolayer. Individual cells are asymmetric, with the apical (mucosal) and basolateral (serosal) surfaces exhibiting different membrane morphology, ionic permeabilities, distribution of enzymes and sensitivity to hormones and drugs<sup>1-4</sup>. This asymmetry is correlated with the

presence of an intact tight junction which encircles the cell at the boundary separating the apical and basolateral surfaces<sup>4-11</sup>. The tight junction seems to form a barrier to extracellular penetration of solute and water across the monolayer<sup>4</sup>.

The mechanisms of development and maintenance of cell asymmetry are unknown. It has been suggested that the tight junction may act as a barrier to lateral diffusion of membrane constituents between apical and basolateral surfaces<sup>4,6,8,9,11</sup>. We report here the results of experiments designed to test that

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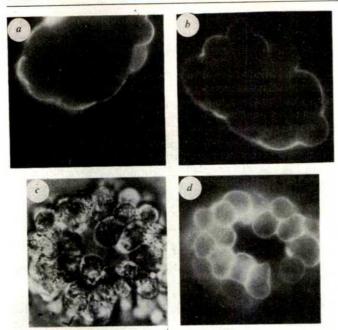


Fig. 1 Fluorescence micrographs of domes of A6 cell monolayers labelled with tetramethyl rhodamine-conjugated wheat-germ agglutinin (a),  $AFC_{16}(b)$  and  $dilC_{16}(d)$ . c, Brightfield photograph of the dome in d. In all cases, the cells were labelled by adding the fluorescent probe to the medium bathing the apical surface of the cell monolayer. In this way, only apical membranes were labelled initially. The plane of focus is part way up a dome of cells. In this geometry, labelling of the apical surface alone appears as a series of arcs, as in a and b, and labelling of the entire plasma membrane appears as complete rings, as in d. The apparent gap in the ring of fluorescent cells in d is due to a cell which was weakly labelled with dilC<sub>16</sub>, as occasionally happens with this probe. Labelling conditions were:  $25 \,\mu \mathrm{g} \,\mathrm{ml}^{-1}$  wheat-germ agglutinin for 20 min;  $10 \,\mu \mathrm{g} \,\mathrm{ml}^{-1}$  AFC<sub>16</sub> for  $10 \,\mathrm{min}$ ;  $5 \,\mu \mathrm{g} \,\mathrm{ml}^{-1}$  dilC<sub>16</sub> for  $5 \,\mathrm{min}$ . × 600.

suggestion. We selectively labelled with a fluorescent probe one surface of a confluent monolayer of epithelial cells grown in culture, and then determined the rate at which the probe diffused laterally in the membrane and whether it could move through the region of the tight junction to the side of the cell not labelled initially (for example, from the apical to basolateral surface). We first measured lateral diffusion coefficients using the technique of fluorescence photobleaching recovery (FPR)<sup>12</sup>, and then monitored the amount of fluorescence on the apical and basolateral surfaces to determine any movement through the tight junction. We found that cell-surface sites labelled with fluorescently conjugated lectins were unable to move through the region of the tight junction and, in fact, were completely

Fig. 2 Molecular structure of the fluorescent lipid probes. AFC<sub>16</sub>, 5-(N-hexadecanoyl)aminofluorescein; AFC<sub>12</sub>, 5-(N-dodecanoyl)aminofluorescein (Molecular Probes); diIC<sub>16</sub>, 3,3'-dihexadecylindocarbocyanine iodide (given by Dr A. Waggoner); NBD-PC, 1-acyl-2-(N-4-nitrobenz-2-oxa-1, 3-diazole)-aminocaproyl phosphatidylcholine (Avanti). AFC<sub>12</sub>, AFC<sub>16</sub> and diIC<sub>16</sub> did not affect transepithelial resistance when added to the apical solution of A6 epithelia grown on a porous support (Millipore filter).

immobile with respect to lateral diffusion. On the other hand, fluorescent lipophilic probes diffused freely, and fell into two categories: those capable of passing the region of the tight junction by lateral diffusion in the membrane, and those incapable of penetrating the tight junction. These results show that epithelial cells are capable of segregating membrane constituents as small as lipids on one side of the tight junction, thus suggesting a mechanism whereby epithelia may maintain different lipid compositions on the apical as opposed to the basolateral membranes<sup>3,13</sup>. As the ability of a lipid probe to pass through the tight junction is also correlated with its access to the inner monolayer of the cell membrane bilayer, we suggest that the tight junction presents an impassable barrier to lipid components only in the outer leaflet of the membrane.

#### Lectin probes

Continuous epithelial cell lines were grown to confluency in culture according to methods published elsewhere 4.14.15. Cell lines used were A6 (derived from toad kidney), LLC-PK<sub>1</sub> (from pig kidney) and MDCK (from dog kidney). These cultures grow with their apical surface uppermost, allowing easy labelling of the apical membrane with fluorescently conjugated lectins. Preparations with exposed basolateral surfaces were obtained by gently detaching part of an intact sheet of cells with a stream of medium from a syringe and folding the flap of cells thus produced back over a glass disk (5 mm diameter, 1 mm high) with the basolateral surface uppermost. This inverted sheet of cells was held in place by a ring clamped around the disk.

Wheat-germ agglutinin and peanut agglutinin lectins were used to label directly both basal and apical surfaces. However, if one surface was labelled, the probe did not redistribute to the other side of the cell, as determined by direct observation with high resolution fluorescence microscopy. It was found that the most convenient geometry for viewing the sidedness of the label was to focus the microscope part way up a dome of cells. Domes are regions of the monolayer which have detached from the dish and bulged upwards due to epithelial transport of solution across the monolayer from apical to basal surface, where it becomes sequestered inside the dome of cells thus produced16. By focusing part way up the dome, apical and basolateral surfaces on individual cells are in focus simultaneously. Thus, if the entire plasma membrane is labelled, each cell in the cross-section of the dome defined by the plane of focus of the microscope will appear as a complete ring of fluorescence. If only one membrane is labelled, incomplete fluorescent rings, or arcs, will be seen. Figure 1a shows such arcs on a dome of A6 cells having apical membranes stained with rhodamine-conjugated wheat-germ agglutinin.

As the lectin-labelled membrane components were unable to penetrate the tight junction, we next determined whether the

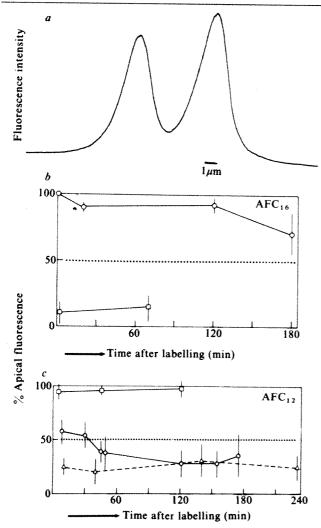


Fig. 3 a, Fluorescence intensity tin arbitrary units) excited by focused krypton ion laser beam (at 531 nm) as the microscope focus was scanned through an A6 cell labelled with diIC<sub>14</sub>. The peak on the left is due to basal membrane fluorescence, and that on the right due to apical fluorescence. b, Mean ( $\pm$ s.d.) per cent fluorescence excited from apical membrane as a function of time after labelling cells with AFC<sub>16</sub>.  $\bigcirc$ , Apical membrane initially labelled;  $\square$ , basolateral membrane initially labelled. The 476 krypton laser line was used to excite fluorescence. c, Mean ( $\pm$ s.d.) per cent fluorescence excited from apical membrane as a function of time after labelling cells with AFC<sub>12</sub>.  $\bigcirc$ , Apical membrane initially labelled;  $\square$ , temperature reduced to 10 °C and apical membrane labelled.

tight junction formed a barrier to lateral diffusion of the label in the membrane. Previous measurements of lateral diffusion of wheat-germ agglutinin binding sites on fibroblasts have yielded diffusion coefficients of  $\sim\!\!2\times10^{-11}$  to  $2\times10^{-10}\,\mathrm{cm^2\,s^{-1}}$  with 80% of the probe freely mobile  $^{17}$ . In contrast, our FPR measurements clearly demonstrated that wheat-germ agglutinin, as well as peanut agglutinin, were completely immobile on both apical and basal membranes. It is thus unnecessary to postulate a diffusion barrier for the maintenance of the asymmetrical distribution of these probes.

The monolayers can be dispersed into single-cell suspensions by chelation of divalent cations, which has been shown to disrupt tight junction filaments in epithelia<sup>4,6,8,9,11</sup>. When this is done after first labelling one side of the monolayer with a lectin, the label spreads over the entire surface of the cell. After spreading, however, FPR measurements show that the probe is still completely immobile with respect to diffusion. Lectin binding sites maintain their relative positions during the spreading and do not inter-mix. Thus, the movement of the lectin while spreading over the entire cell surface after disruption of the monolayer apparently does not involve diffusion.

#### Lipid probes

We next investigated the behaviour of several fluorescent lipophilic probes introduced exogenously to the cell membranes. These probes (see Fig. 2) fell into two groups with respect to their labelling patterns: those which penetrated the tight junction (dilC<sub>n</sub> for n = 14, 16, 18, 20 and AFC<sub>12</sub>), and those which did not penetrate the tight junction (NBD-PC and AFC<sub>16</sub>). An example of domes labelled with AFC<sub>16</sub> is shown in Fig. 1b, where the scalloped pattern due to one-sided labelling is evident. In contrast, Fig. 1d shows a dome labelled with dilC<sub>16</sub>, which labels the entire cell membrane. As was the case with the lectins, all the non-penetrating lipid probes spread over the entire cell surface when the cell monolayer was disrupted by chelation of divalent cations.

We were also able to observe directly diffusion of the diIC<sub>n</sub> probes through the tight junction. By carefully manipulating the x-y position of the microscope stage, we manoeuvred a single cell (labelled with one of the diIC<sub>n</sub> probes) on the side of a dome (as in Fig. 1d) under an intense, focused laser beam so that all the fluoresence from either the apical or basolateral surface was bleached. Then, under normal fluorescence illumination, we observed fluorescence returning to the bleached membrane due to diffusion from the unbleached part of the cell on the other side of the tight junction. The fluorescence was replenished in a few minutes, which is as expected from the measured diffusion coefficient (Table 1). If the entire cell was bleached, no replenishment occurred. There was no transfer of the probe from neighbouring cells.

As the barrier to penetration between apical and basolateral membranes seems to occur at the tight junction, we tested whether epithelial cell lines having different permeability characteristics for aqueous solutes would exhibit different labelling patterns with the lipophilic probes. In many cases, 'leakier' epithelia have less extensive fibrillar networks forming the tight junction than 'tight' epithelia'. We found the labelling patterns for the probes in Fig. 2 to be identical in A6 cells (tight epithelium: trans-epithelial resistance =  $5,000~\Omega~cm^2$ ), MDCK cells (leaky epithelium: resistance =  $100~\Omega~cm^2$ ) and LLC-PK<sub>1</sub> cells (intermediate: resistance =  $400~\Omega~cm^2$ ) and LLC-PK<sub>1</sub> cells (intermediate: nesistance =  $400~\Omega~cm^2$ )

A more quantitative measure of the distribution of the fluorescent probes was obtained by measuring fluorescence intensities from apical and basal membranes separately on individual cells. The focused laser beam was used to excite fluorescence from a small spot (as in the FPR measurements) as the microscope focus was slowly scanned through the centre of a cell. A peak of fluorescence appeared, first as the apical, then as the basal membrane passed through focus (Fig. 3a). Figure 3b shows the time dependence of the fluorescence distribution thus measured for AFC<sub>16</sub>. This probe clearly does not penetrate significantly from apical to basal membrane, or vice versa, over time periods of a least 2h. AFC<sub>12</sub>, on the other hand, distributes

Table 1 Lateral diffusion coefficients of lipid probes in A6 cell membranes

Probe	Apical	Basal	Pass tight junctions
diIC14	$9.7 \pm 3.6 (17)$	$11.9 \pm 2.3 (14)$	+
diIC <sub>16</sub>	$7.9 \pm 3.4 (18)$	$10.2 \pm 5.6 (19)$	+
AFC <sub>12</sub>	$13.3 \pm 4.6 (10)$	$11.2 \pm 3.2 (9)$	+
AFC <sub>16</sub>	$4.2 \pm 1.8 (18)$	ND	
NBD-PC	$2.9 \pm 1.9 (33)$	$4.2 \pm 1.7$ (28)	une.

Values shown are the mean lateral diffusion coefficient  $(D)\pm s.d.$   $(\times 10^9 \text{ cm}^2 \text{ s}^{-1})$ . The number of cells measured is shown in parentheses. ND, not determined. The values for D are not corrected for the non-planar membrane geometry due to the presence of microvilli, which probably accounts at least in part for the higher values usually obtained on the basal membranes, which are non-villous.

so that  $\sim 30\%$  of the fluorescence finally localizes on the apical membrane, regardless of whether the apical or basal membrane was labelled initially (Fig. 3c). In addition, the distribution of AFC<sub>12</sub> is temperature dependent, with no passage from apical to basal membranes occurring if the cells are kept below  $10^{\circ}\text{C}$ —this cannot be explained by a low temperature immobilization of AFC<sub>12</sub>, as FPR measurements at this temperature showed that AFC<sub>12</sub> was still free to diffuse, although at a rather slower rate (data not shown).

The time course of the AFC $_{12}$  distribution has another curious feature. Within minutes after labelling the apical membrane, the probe redistributed so that 50-60% of the fluorescence localized on the apical membrane. Within the next 30-60 min this proportion gradually changed until only 25-30% of the fluorescence lay on the apical surface (Fig. 3c). This is not a trivial geometric effect due to the presence of more membrane on the basal surface. In fact the opposite is the case  $^{14}$ . The same apportioning of fluorescence occurred when the intact cell monolayer was detached and turned over and the basolateral membranes labelled initially (Fig. 3c). In contrast, the diIC $_{16}$  maintains 50-60% of its fluorescence on the apical membrane

It is possible that the lipid compositions of the apical and basolateral membranes are different, and that  $AFC_{12}$  prefers to 'dissolve' or partition into the lipids of the basolateral membrane. This is plausible, as differences in lipid composition between apical and basolateral membranes of epithelia have been reported<sup>3,13,21-23</sup>. Another possibility is that the probe's fluorescence is partially quenched at the apical surface, perhaps, for example, by low pH. We have no evidence to exclude either possibility.

# Basis of selectivity of the tight junction barrier

The molecular basis for the selectivity of the tight junction barrier to penetration of lipid probes is of primary importance both because of what it may reveal about the structure of the tight junction itself, and because of the implication that it may permit the cell to maintain different lipid compositions on the apical as opposed to the basolateral membranes. From the structures shown in Fig. 2, it is evident that molecular charge cannot be the sole determining factor, as there are positively and negatively charged probes which do penetrate (diIC, and AFC<sub>12</sub>), and negatively charged and uncharged probes which do not penetrate (AFC<sub>16</sub> and NBD-PC) the tight junction. The degree of partition of the probe between aqueous and membrane phases is also probably not important in determining whether the probe penetrates the tight junction. AFC12 and NBD-PC are the two probes with the highest aqueous phase partitioning, but they exhibit opposite behaviour with respect to passing through the tight junction, as do AFC<sub>16</sub> and the diIC<sub>n</sub>, which partition least in the aqueous phase.

It is interesting that  $AFC_{12}$  and  $AFC_{16}$ , which differ only in fatty acyl chain length, show opposite behaviour regarding penetration of the tight junction, which may indicate that interactions in the hydrophobic region of the membrane are important. In this regard, Table 1 shows that the lateral diffusion coefficients of the lipid probes are predictive of whether or not the probe penetrates the tight junction: all probes that can pass through the tight junction have lateral diffusion coefficients greater than  $\sim 8 \times 10^{-9}$  cm<sup>2</sup> s<sup>-1</sup>, whereas all those that are incapable of penetrating the junction have lateral diffusion coefficients  $< 4 \times 10^{-9}$  cm<sup>2</sup> s<sup>-1</sup>. Apparently, the two classes of lipid probes interact with the lipid bilayer in significantly different ways, as reflected by their lateral diffusion coefficients. This difference may provide the basis for whether a probe can penetrate the tight junction or not.

In view of the possibility that the two classes of lipid probes interact differently with the cell membrane, it is important to assess whether the probes are in fact inserted into the membrane lipid bilayer. From the molecular structures in Fig. 2 one would expect, on energetic grounds, insertion of the hydrocarbon chains into the membrane bilayer. On the other hand, it is

conceivable that a probe may form micellar structures which adhere to the cell surface and prevent true incorporation of the probe into the membrane. We consider this unlikely for several reasons. First, the measured diffusion coefficients are all in the range observed for lipid-like molecules in cell membranes<sup>24–29</sup>. In addition, the conjugated bridge of dilC<sub>18</sub> has been shown to be aligned parallel to the plane of the membrane when the probe labels erythrocyte ghosts, exactly as expected if the hydrocarbon tails are inserted into the membrane bilayer<sup>30</sup>. Finally, our fluorescence intensity and spectral measurements have shown that the probes are in a much more hydrophobic environment when labelling cells than when dispersed in aqueous media<sup>31</sup>, so that the possibility of probe micelles adhering to the cell surface is unlikely.

We feel that a possible basis for the observed selectivity of the tight junction lies in which leaflet of the membrane bilayer is occupied by a given probe; those probes which label the outer leaflet only do not pass through the tight junction, whereas those which can 'flip-flop' to the inner leaflet do pass through it. Phospholipids have been shown to flip-flop between membrane leaflets at very low rates in bilayer membranes <sup>32-34</sup>, and it seems reasonable to assume that NBD-PC behaves similarly. The smaller polar 'head group' region of the diIC<sub>n</sub> may allow it to pass more rapidly between inner and outer membrane leaflets, and the shorter acyl chain of AFC<sub>12</sub> relative to AFC<sub>16</sub> may facilitate its flip-flop, a process which is inhibited at low temperature.

Experimental evidence in support of this hypothesis has come from measuring the accessibility of the various membrane-bound probes to quenching from outside the membrane<sup>31</sup>. Using iodide to quench diIC<sub>16</sub> fluorescence and anti-fluorescein antibodies to quench AFC<sub>12</sub> and AFC<sub>16</sub> fluorescence, our data show that (1) diIC<sub>16</sub> seems to have access to both leaflets of phospholipid vesicle membranes; (2) AFC<sub>16</sub> is present almost exclusively in the outer membrane leaflet of labelled cells; and (3) AFC<sub>12</sub> can 'flip-flop' between membrane leaflets at 20 °C, but not at 5 °C. These results imply that the tight junction may form a barrier to passage of lipids only in the outer membrane leaflet. Thus, differences in lipid composition between apical and basolateral membranes could reflect differences in the compositions of the outer monolayer lipids.

#### Conclusion

We have studied the mechanism of maintenance of cellular asymmetry in epithelial cells grown in culture by directly observing whether fluorescent probes applied to either the apical or basolateral membranes spread to the unlabelled surface. Lectins which were bound to either the apical or basolateral membrane remained segregated there and were immobile with respect to lateral diffusion in the membrane. Some lipid probes introduced exogenously into the membrane were able to penetrate to the unlabelled side of the cell monolayer, whereas other lipid probes were not. All lipid probes were freely mobile in the membrane, although those which were able to spread over the entire cell had lateral diffusion coefficients at least twofold greater than those which could not. The barrier to spreading of the probes to the unlabelled surface was at the region of the tight junction. Chelation of divalent cations, which disperses the monolayer and disrupts tight junction filaments, allowed spreading of all the impermeant probes over the cell surface, although in the case of the lectins the spreading apparently did not involve free diffusion. The selectivity of the tight junction region to passage of the lipid probes was identical in monolayers of epithelial cell lines having markedly different 'leakiness' to aqueous solutes.

These results directly demonstrate that the region of the tight junction can act as a barrier to lateral diffusion of membrane constituents as small as lipids, potentially enabling epithelia to segregate at least some lipids to the apical or basolateral membrane and thus maintain an asymmetric lipid distribution. Fluorescence quenching experiments support the hypothesis that the lipid probes that have access to the inner leaflet of the

cell bilayer are capable of passing the tight junction, but those present exclusively in the outer leaflet are blocked by the tight

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# Probable optical counterpart of a y-ray burster

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I describe here  $1.6 \times 10^7$  s (over half a year) of optical monitoring of three γ-ray burst positions using the collection of archival plates at the Harvard College Observatory. The search has uncovered the probable optical counterpart for the 19 November 1978  $\gamma$ -ray burster on a blue emulsion plate exposed in 1928. The optical flash observed on the 1928 plate has  $m_B \sim 3$ , for an assumed duration of 1 s. Optical searches indicate that the absolute magnitude of the  $\gamma$ -ray burst system at quiescence is fainter than 13. A recurrence rate of  $\sim 10^{-7.5}$  s<sup>-1</sup> is found from the  $\gamma$ -ray and optical data. A recurrence rate this high rules out any model which uses a collision between a neutron star and an asteroid-like body as well as any model which requires accretion from the interstellar matter onto a neutron star.

Since the discovery of cosmic y-ray bursts1, there have been many observations of these phenomena2, but so far no celestial object has been positively identified with any y-ray burster at other than y-ray energies3-7. In order to make an identification, an interplanetary network of satellites with γ-ray detectors has been established, so as to locate the direction of the  $\gamma$ -ray burst by triangulation<sup>2,3</sup>. Although this network has determined error boxes ~1 arc min in radius for several bursters<sup>3,8-11</sup>, no convincing optical<sup>3,12,13</sup>, radio<sup>14</sup> or X-ray<sup>10,15</sup> counterparts have been proposed.

Grindlay, Wright and McCrosky<sup>7</sup> have shown that a simple extrapolation of the observed y-ray spectrum implies that the sources would be optical flares with  $m_v \sim 0$ . This failure to detect optical flashes from two  $\gamma$ -ray bursts in the early 1970s allowed them to set limits on the brightness of the two events as  $m_{\rm v} > 3.0$  and 3.8 mag for an assumed duration of 1 s. The advantage of their work over that reported here is that a  $\gamma$ -ray burst was seen during their exposure.

Both theoretical and observational arguments suggest that  $\gamma$ -ray bursters are recurring phenomena. Jennings and White<sup>16</sup> use the observed  $\log N(>S) - \log S$  relation to deduce that the  $\gamma$ -ray recurrence rate must be  $>10^{-12} \, \mathrm{s}^{-1}$  per burster. For thermonuclear flash models<sup>17</sup>, the recurrence rate is predicted to

be between  $10^{-10}$  and  $10^{-7} \, \mathrm{s}^{-1}$ . The observational argument is based on several bursts, closely spaced in time, which seem to have identical positions<sup>18-20</sup>.

I have carried out a search of three precisely known  $\gamma$ -ray burst positions<sup>8-10</sup> on plates in the archival collection of Harvard College Observatory (see Table 1). On one of the plates for the 19 November 1978 field<sup>10</sup>, a new 10-mag star appears in the γ-ray error box. The plate is MF12559 taken on 17 November 1928 in South Africa. It is the fourth of six identical 45-min exposures taken in succession. Figure 1 is a reproduction of the third and fourth plates in the series. The 'new' star does not appear on any of the other five plates (all of which have a limiting magnitude of  $\sim 15$ ).

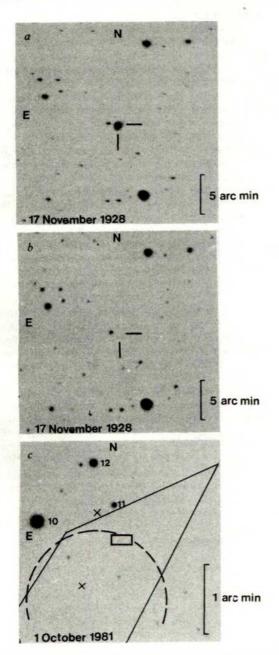
Microdensitometry provides strong evidence that the 'new' star image was formed by a brief flash of light which came through the telescope. The images on the fourth plate are trailed by 17 arcs, but the image of the 'new' star is not noticeably trailed, as would be expected if it were a short (≤10 min) optical flash. Figure 2 shows the profiles of the burst image and of two nearby normal star images in a direction perpendicular to the trailing. The asymmetry is due to coma, which implies that the burst image must have been formed by light that passed through the telescope optics and that plate defects and light leaks are not a possible explanation for the burster candidate. The profiles of normal stars as well as the candidate show a break at ~110 µm. which is further confirmation that the light that formed the burst image followed the same path as normal starlight.

The slope of the burst image profile in Fig. 2 is less than the slope of a normal star profile, which can be attributed to the failure of the law of reciprocity in photographic emulsions. In the relevant range of radial distances (r), the intensity (I) of light that reaches the plate will fall off as  $r^{-\eta}$  where  $\eta$  is some constant<sup>21-23</sup>. According to Schwarzschild's law of reciprocity failure  $^{24-26}$ , the photographic density (D) is proportional to  $I^{a}T$ , where q is a function of the duration of exposure (T). Thus and the slope on a log D versus log r plot is  $(-\eta q)$ . For a short flash, q is  $\leq 1$  (ref. 26). For a 45-min exposure, q is typically<sup>26</sup> 1.25, so that the normal star profile is expected to be steeper than the profile from a quick flash. Unfortunately, this argument cannot be made more quantitative because the emulsion type and developing conditions are unknown.

The 1928 image may have been formed by a 2-mag meteor viewed exactly head-on. The probability<sup>27</sup> of detecting a meteor in a y-ray error box on any plate used in this study is  $\sim 10^{-2}$ . Microdensitometry shows that the 1928 image is not trailed,

with an upper limit of 3 arcs trailing. The probability that a meteor will approach within this narrow tolerance of the head-on direction is  $10^{-8}$ . The joint probability of both seeing a meteor in a  $\gamma$ -ray burst error box and having that meteor appear head-on is  $\sim 10^{-10}$ .

The position of the image (1 h 16 min 25.8 s ± 0.6 s and  $-28^{\circ}$  51' 1.2" ± 2.1" measured with a Mann measuring machine) is inside the  $\gamma$ -ray error-box<sup>10</sup> but is not consistent with any observed radio sources<sup>14</sup> (see Fig. 1). The position is, however, inside the reported error-box of a possible (3.5 $\sigma$ ) X-ray source<sup>10</sup>. Recently, M. H. Liller took deep B and V 4-m plates of this region at CTIO. Both plates show faint stellar images ( $m \sim 23$ ) at the same location in the optical error box



**Fig. 1** Three views of the 19 November 1978 γ-ray burst field. a, Plate MF12559, with tick marks indicating the burst image. The burst image has a different shape from that of a normal star; a short flash is not trailed and has a more gradual fall off of density with distance from the centre of the image. b, Plate MF12558, which was taken 45 min before MF12559. c, A V plate taken by M. H. Liller on the 4-m telescope at CTIO. The image inside the  $2\sigma$  1928 optical positional error rectangle (centre) cannot be seen. The  $\gamma$ -ray error region 10 (solid lines) and possible X-ray error circle 10 (dashed lines) are also shown. Radio sources 14 are indicated with crosses. Three stars are numbered as in ref. 12.

Table 1 Exposure versus burst limiting magnitude

Limiting mag.	Hours of exposure which reach the limiting mag						
for detecting a 1 s burst	19 November 1978	5 March 1979	6 April 1979	Total			
3.0- 3.9	671	2,270	1,509	4,450			
4.0- 4.9	635	2,148	1,422	4,205			
5.0- 5.9	560	1,716	1,198	3,474			
6.0- 6.9	407	961	710	2,078			
7.0- 7.9	289	373	269	931			
8.0- 8.9	174	149	71	394			
9.0- 9.9	96	26	23	145			
10.0-10.9	34	1	7	42			

(1 h 16 min 25.99 s  $\pm$  0.02 s and  $-28^{\circ}$  51' 2.3"  $\pm$  0.3", 1950.0). Using a PDS microdensitometer, I find the images to be 3.2 $\sigma$  and 4.9 $\sigma$  above noise for the B and V plates respectively. A search by G. R. Ricker (personal communication) has, however, failed to locate this star with a CCD camera (the MASCOT) on the McGraw-Hill 1.3-m telescope ( $m \ge 24$  at  $2\sigma$  for 4,000-7,000 Å). Because of the uncertain thresholds and different band-passes, the question of the reality of this stellar image is not yet established. Deeper searches with the MASCOT have been carried out and the data are being analysed.

The magnitude of the image on the 1928 Harvard plate is uncertain because the profile of the burst image differs from a comparison star's profile. Exact modelling is impossible because the plate characteristics and burst duration are unknown. However, a reasonable model shows  $m_{\rm B} \sim 3$  for a burst lasting 1 s, which corresponds to a time-integrated flux of  $4\times 10^{-7}\,{\rm erg}\,{\rm cm}^{-2}$  above the atmosphere<sup>27</sup>. Assuming that the 1928 and 1978 bursts were similar,  $L_{\gamma}(>30~{\rm keV})/L_{\rm opt}(B~{\rm band})$  is 800 and the energy flux density is proportional to  $\nu^{0.6}$ .

Three other plates show density enhancements in the  $\gamma$ -ray burst error regions. A plate exposed in 1948 shows a 'new' star in the 19 November 1978 optical error box, and two plates (from 1932 and 1936) show 'new' stars in the 6 April 1979  $\gamma$ -ray error box. Because these three images are near the limiting magnitude of their respective plates a detailed analysis of the image profile is impossible.

Table 2 summarizes the characteristics and total exposure for the seven series of plates used in this study. For each plate, I estimated the magnitude of the faintest star image detectable. Burst-limiting magnitude' for each plate was derived from the known exposure times, an assumed burst duration, an approximate knowledge of the reciprocity failure, and the stellar limiting magnitude. The burst-limiting magnitude indicates how bright an optical flash of given duration must be to ensure detection on a given plate. An optical flash inside the nebulosity of N49 would be harder to detect, so the 'burst limiting magnitude' should be decreased by up to 1 or 2 mag for the 5 March 1979 field. I assume that the optical burst lasts 1 s as this is comparable to the durations of  $\gamma$ -ray bursts. Table 1 shows the total exposure for each of the three  $\gamma$ -ray positions that could lead to the detection of a burst of a given magnitude.

The steady optical flux from the burster can severely limit the types of stellar components which can be in the burster system. The CTIO 4-m plates of Liller indicate that the quiescent optical counterpart is fainter than ~23 mag. Jennings and White 16 show that the γ-ray bursters are probably not extragalactic nor are they distributed in a halo around our Galaxy. They deduce that the most likely distribution of γ-ray bursters is a disk population with a scale height of ~400 pc. In this case, the distance to the 19 November 1978 burster must be ≤1 kpc, because of its position near the south galactic pole. The limits on both the apparent magnitude and the distance yield a lower limit of 13 mag for the burster's absolute magnitude. Only three known classes of stars (white dwarfs, neutron stars and late M main sequence stars) satisfy this limit, and hence could be components of the burster system. Furthermore, if the ~23 mag star on Liller's plate is the optical counterpart of the burster, then this cannot be a lone neutron star. This is because a lone neutron star has an absolute

Table 2 Characteristics of Harvard plates

Plate series	Aperture (cm)	Plate scale (arc s mm <sup>-1</sup> )	Dates	Total exposure (h)	Total no. of plates	Median limiting mag.	Median limiting mag. for 1 s burst
Α	61.0	60	1896-1950	68	¥ 50	16.5	8.2
AM/AX	3.8	600	1901-1953	2,689	2,465	14.1	5.8
В	20.3	179	1889-1948	198	414	15.3	8.1
Damon	4.1	580	1970-1979	75	50	15.2	6.9
MF	25.4	167	1918-1950	210	299	16.4	8.9
RB	7.6	391	1928-1950	1,210	857	14.8	6.5

magnitude >23 (assuming the neutron star radiates as a black body<sup>28</sup>), and would have to be placed at such a close distance as to violate the  $\log N$  (>S) -  $\log S$  observations. We note (D. O. Lamb, personal communication) that the class of y-ray burst models which invoke an energy supply internal to a neutron star<sup>29</sup> do not require that the neutron star have a companion.

The value for the burster recurrence rate can be estimated. (1) From detailed modelling of the log N(>S) - log S relation. Jennings and White<sup>16</sup> show that the recurrence rate must be between  $10^{-12}$  and  $10^{-1}$  s. (2) Let  $N_T$  be the total number of γ-ray burst sources which are accessible to near-Earth detectors with a threshold  $f_0$ , and let R be the number of  $\gamma$ -ray bursts observed with flux  $> f_0$  which arrive at Earth per second. The average recurrence rate will be just  $R/N_T$ .  $N_T$  must be greater than the total number  $(N_0)$  of non-overlapping error boxes determined for bursts with a flux greater than  $f_0$ . From the data base of Mazets and Golenetskii<sup>20</sup>, with  $f_0 \sim 10^{-6}$  erg cm<sup>-2</sup>,  $R \sim$  $10^{-5.8} \,\mathrm{s^{-1}}$  and  $N_{\mathrm{T}} > N_{\mathrm{0}} > 35$ , the recurrence rate must be  $<10^{-7.3}$  s<sup>-1</sup>. (3) With a total observing time of  $10^{7.3}$  s, the data from Mazets and Golenetskii<sup>20</sup> suggest two and possibly more cases of a  $\gamma$ -ray burster recurring. These cases suggest the burst

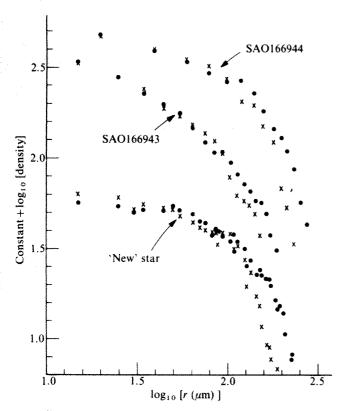


Fig. 2 Profiles along the north-south axis of the burst image and two nearby normal stars. The densities north (south) of the image centre are indicated with ×(•). The differences between the north and south profiles are due entirely to coma, because the scan direction is perpendicular to the direction of trailing. The profile of the burst image has been lowered by 0.6 in the logarithm of density for clarity. The zero of the density scale is set to the density of the sky background.

rate is  $\sim 10^{-7} \, \text{s}^{-1}$ . (4) One (and possibly as many as four) optical bursts were seen during a monitoring time of 10<sup>7.2</sup> s, which implies a recurrence rate of order  $10^{-7.2}$  s<sup>-1</sup>. From all the evidence, a reasonable estimate of the burster recurrence rate is  $\sim 10^{-7.5} \,\mathrm{s}^{-1}$  (one burst per year).

This high recurrence rate precludes the hypothesis where an asteroid (or comet) impacts onto a neutron star<sup>30</sup>, which predicts typical recurrence rates on the order of  $10^{-13}$  s<sup>-1</sup> (refs 31, 32). A popular class of models envisions a thermonuclear explosion on a neutron star surface. For a typical burst energy <sup>16</sup> of 10<sup>39</sup> erg and mass-to-energy conversion<sup>33</sup> of 10<sup>19</sup> erg g<sup>-1</sup>, the neutron star will need to accrete 10<sup>20</sup> g of fuel for each burst. To transfer this amount of mass every  $\sim 10^{7.5}$  s, the average accretion rate is many orders of magnitude higher than is obtainable by accretion from the interstellar medium alone. If this accretion rate remains constant, then a source 100 pc distant would imply a steady X-ray flux of  $\sim 10^{-10}$  erg s<sup>-1</sup> cm<sup>-2</sup>. Observations with the Einstein Observatory<sup>3</sup> have placed upper limits of  $\sim 10^{-12} \, \text{erg s}^{-1} \, \text{cm}^{-2}$  on the steady X-ray flux from the three  $\gamma$ -ray bursters listed in Table 1. If the thermonuclear explosion model and the physical values are correct, this discrepancy can be explained by assuming either that most of the accretion energy is not radiated in the X-ray or that the accretion is not steady.

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# A four-hour orbital period of the X-ray burster 4U/MXB1636-53

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It has been generally accepted that X-ray burst sources and other non-bursting 'galactic bulge X-ray sources' are low-mass close binary systems (for a review see ref. 1). The companion stars of the transient burst sources Aql X-1 (= Aql MXB = 4U1908+00) and Cen X-4 (= XB1455-31) are of spectral types G7-K3V (ref. 2) and K3-7V (ref. 3), respectively. The companion of the non-bursting transient source A0620-00 is of spectral type K4-5V (refs 4, 5). If these stars fill their Roche lobe, which is likely, the orbital periods are in the range 5-8 h. There is some evidence from X-ray observations alone that the orbital period of Cen X-4 is ~8 h (ref. 6). From an analysis of their average optical properties, one of us (J.vP. ref. 7) estimated that the typical values of the companion star masses and orbital periods of this class of low-mass X-ray binaries are ~0.6  $M_{\odot}$  and ~6 h, respectively. The orbital period recently suggested for the burst source 4U/MXB1735-44 (ref. 8) is in accordance with this general picture. We here report variations in the persistent optical flux of 4U/MXB1636-53 which seem to vary periodically with a period near 4 h.

During June-August 1979 and 1980 extensive optical observations were made of the X-ray burst source 4U/MXB1636-53 (see refs 9, 10 for properties of the faint blue counterpart  $(B\sim18)$  of this source). Some of these observations were made simultaneously with the Japanese X-ray observatory Hakucho. Results on correlated optical/X-ray bursts and other optical bursts will be reported elsewhere  $^{11,12}$ .

The observations were made with the Danish 1.5-m telescope at the European Southern Observatory (ESO) at La Silla. During the 1980 observations a double-channel photometer was used with no filter. The combination of atmospheric transmission, optics and photomultiplier sensitivity defines a 'white light' passband with an effective wavelength of  $\sim 4,300 \, \text{Å}$  and a full width at half-maximum of  $\sim 1,900 \, \text{Å}$ . The telescope was guided using an autoguiding system which kept the star centred to  $\leq 1$  arc s. In the channel used for measurements of 4U/MXB1636-53, a 5 arc s diaphragm was used. The reference channel which used a 20 arc s diaphragm was set on a star  $\sim 3$  mag brighter a few arc minutes away from the main channel. The ratio between stellar signal and sky brightness was similar for the two channels (1.5 and  $\sim 1.2$ , respectively).

We observed 4U/MXB1636-53 on 11-13 July 1980. The data trains span  $\approx 7$  h each night. The nights were dark, photometric and the seeing was less than 2 arc s. Figure 1 shows the observed count rates binned into 512-s time slots, together with similar data for the reference channel. The latter is constant to within  $\pm 2\%$  throughout each night. Clearly, however, the persistent optical flux of 4U/MXB1636-53 is varying. As the ratio of stellar to sky signal is approximately equal in the two channels, this variation cannot be due to changes in the sky brightness. Such changes would result in a similar variation (approximately same percentage) in the reference signal and that is not observed. Furthermore, the relatively good seeing in combination with the 5 arc s diaphragm and the high-quality autoguiding of the star (see above), exclude the possibility that

the variation is due to variable signal contamination by a star of similar brightness about 7 arcs north of 4U/MXB1636-53. The data suggest that the variability may be periodic. Five broad maxima occur in the optical brightness near 11 July, 0200 and 0615 UT, 12 July, 0100 and 0430 UT and near 13 July, 0315 UT. These maxima are consistent with a single period of approximately 4 h.

To establish a more accurate period, we have folded the data with trial periods between 2 and 6 h, and searched for periods where  $\chi^2$  of the data points, relative to the average curve (divided into 20 phase bins), showed a minimum. Before folding the data, the overall nightly average counting rate was subtracted. Three possible periods turned up (see Fig. 2) 3.28 $\pm$ 0.05 h, 3.78 $\pm$ 0.05 h and 4.42 $\pm$ 0.05 h, corresponding to 15, 13, and 11 cycles between the maxima on 11 July, 0200 UT and 13 July, 0315 UT, respectively. Figure 3 shows the folded count rates (as described above) for the period of 3.78 h. The amplitude is ~25% of the average signal (sky background subtracted).

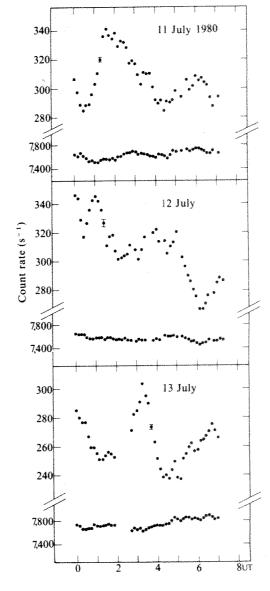


Fig. 1 Count rates, including sky background, for 4U/MXB1636-53 and the reference star (upper and lower set of points in each panel) as observed on 11, 12 and 13 July 1980. Each point represents the average of 10 integrations of 51.2s each. Typical mean errors on the averages of these 10 values are indicated in the figure by one error bar in each panel. The error bars in the reference star data have approximately the size of the dots. The blue magnitude corresponding to the average sky-corrected counting rate is  $B \sim 18.5$ .

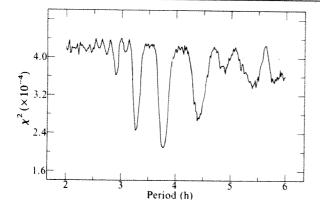


Fig. 2  $\chi^2$  (relative to the average folded curve divided into 20 phase bins) as a function of the folding period. The three deep minima correspond to periods of 3.28, 3.78 and 4.42 h, respectively.

We observed 4U/MXB1636-53 again on 1 August 1981, 0140 UT until 0445 UT, again using the Danish 1.5-m telescope. The photometer used was operated as a single channel instrument. The detector was an RCA C31034A photomultiplier tube illuminated through a 5-mm liquid CuSO<sub>4</sub> filter. A 9 arc s diaphragm was used. The observations were repeatedly interrupted to measure the sky background and the brightness of two nearby companion stars. Photometric conditions were good, with seeing of less than 2 arc s. To avoid any possible contamination of the signal by light from the star  $\sim$ 7 arc s north of 4U/MXB1636-53, the centre of the diaphragm was placed about 1.5 arc s south of 4U/MXB1636-53.

Figure 4 shows the ratio of the counting rates (sky background subtracted) of 4U/MXB1636-53 to one of the reference stars. A smooth modulation of the brightness of 4U/MXB1636-53 is clearly visible, with a shape that is consistent with the average variation found in the 1980 observations (see Fig. 3). Clearly the data are insufficient to decide between the above three allowed time periods.

If any of these three possible periods is the orbital period of 4U/MXB1636-53, then the mass of its companion star can be estimated if the following assumptions are made (see refs 13, 14). (1) The companion star fills its Roche lobe. This is likely in view of the rate of mass transfer needed to power the persistent X-ray emission of 4U/MXB1636-53. (2) The companion star is on the main sequence.

The orbital period, P, is then uniquely determined by the mass  $M_c$  of the companion star according to the relation  $P = 3.14 \times 10^4 \times (M_c/M_\odot)$  s. Using this relation and the above

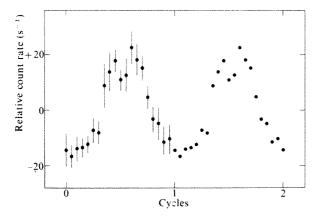


Fig. 3 Average light curve of 4U/MXB1636-53 during 1980, 11, 12 and 13 July, for a folding period of 3.78 h. Before folding the data, the average counting rate, as observed during each night, has been subtracted. The error bars indicate the mean errors of the average values in the phase bins. For convenience the same data have been plotted twice.

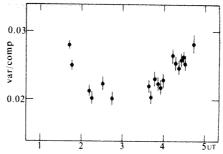


Fig. 4 Ratio of the counting rates (sky-subtracted) of 4U/MXB1636-53 (var) to that of one of the comparison stars (comp). The corresponding ratio of the two comparison stars used is constant to within  $\pm 2\%$ . The data were taken on 1 August 1981.

period of  $\sim$ 4 h, we find a mass of  $\sim$ 0.4 $M_{\odot}$  (corresponding to an M0 V star) for the companion of 4U/MXB1636-53.

If we identify the period in the optical brightness variations of 4U/MXB1636-53 with the binary period, a major deviation from axial symmetry must be present in the system. Within the framework of the low-mass X-ray binary model we can exclude the following possible causes of the brightness modulation.

(1) Eclipses of the accretion disk by the companion star (assumed to fill its Roche lobe). They would result in a much narrower minimum in the light curve than is actually observed (see Fig. 3). (2) As the companion star contributes only of the order of 1% or less to the total optical brightness of the system<sup>7</sup>, changes in its visibility cannot explain the observed brightness modulation either. In particular double-wave ellipsoidal variations (with a corresponding period near 8 h) can be excluded. (3) The modulation cannot be the result of eclipses of a hot spot (where matter is injected into the disk), as observed for some cataclysmic variables14. The luminosity of such a hot spot is expected to be approximately independent of the nature of the compact star at the centre of the disk (white dwarf or neutron star). Because the optical luminosity of low-mass X-ray binaries is a factor of  $\sim 10^2$  larger than that of cataclysmic variables this spot would therefore only contribute of the order of 1% of the total optical brightness of the system.

The disk thickness (seen from the compact object), estimated on the basis of the average optical and UV properties, is ≥6° (ref. 7). In a binary system with a mass ratio q = 0.3 and total disk thickness ≥6°, the solid angle subtended by the non-shielded part of the companion star (as seen from the compact object) is -30% of that of the disk. Thus, if the accretion disk in 4U/MXB1636-53 has a total thickness of about 6° and is not thicker, a significant contribution to the optical brightness variation might be due to the heating by X rays of the companion star and the observed modulation could then be due to the orbital motion of the companion star. It may be that the accretion disk in this system is much thicker than 6° (see the suggestion in ref. 15). For total disk thickness of  $\sim 30^{\circ}$  the optical companion would lie entirely in the X-ray shadow of the disk and this could naturally explain why X-ray eclipses are, in general, not observed in these systems<sup>1,15</sup>. If such a thick disk is present in 4U/MXB1636-53, we suggest that this disk is not axially symmetric, perhaps being thicker where matter is injected into the disk. A similar suggestion has been made for the source 2A1822-37 (refs 16, 17). The brightness modulation could then be the result of the azimuthal dependence of the surface brightness of the disk in combination with the phase dependence of the angle at which the different parts of the disk are viewed.

If this is the case and also if the observed modulation is due to X-ray heating of the companion, we expect a similar modulation with the orbital period of the average ratio of optical to X-ray burst energies. Clearly we then expect that the observed delay between optical and X-ray bursts will also vary with the ~4 h period.

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# Intrinsic variations of the double quasar 0957+56 AB

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Spectroscopic evidence strongly suggests that the two quasars 0957+56 A and B are images produced by the gravitational lensing of a single distant quasar (at z=1.41) by a giant elliptical galaxy and its attendant cluster (at z=0.36)<sup>1-6</sup>. It has also been suggested that the quasar is an intrinsic variable 7.8. I report observations that show a variation of ~1 mag in both components and behaviour typical of intrinsically variable quasars with similar radio structure. The near constancy of the magnitude difference between the components at several epochs despite overall variations favours all the variations being intrinsic to the quasar and also supports the gravitational lens hypothesis. Recent variations of component B1 limit the time delay between the images to between 2 and 24 yr.

In an analysis of the imaging of the double quasar Young et al.5 suggested that three images are formed. Two images A and B1 lie 5 and 1 arc s respectively on either side of a faint elliptical galaxy and correspond to the objects for which photometry and spectrophotometry have been reported. The weak third image B2 is so far undetected and thought to be coincident with the galaxy. Photometry of components A and B1 were obtained from the Palomar Observatory Sky Survey (POSS) and the 26-inch refractor at Herstmonceux. The new photographic B band observations are combined with magnitude differences from photometry and spectrophotometry given by various authors: despite the variety of systems used the observations should be directly comparable. Details of the reductions are given in Fig. 1 legend.

Figure 1 shows the light curve of the double quasar between April 1979 and June 1981. The Herstmonceux photometry is consistent with component A being constant and Young et al. also found A constant before the Herstmonceux observations at B = 17.6. The observations of component B1 are in excellent agreement and show that from April to December 1979 both components were constant with  $B1 \sim 0.35$  mag fainter than A. Between December 1979 and June 1980 B1 brightened by ~0.45 mag and between June 1980 and June 1981 it faded slightly. If this downward trend continues component B1 will return to its previous luminosity by ~April 1982. Notice that the rise and decline do not have the same slope.

Although all the observations of component B1 are to some extent contaminated by the elliptical galaxy and the weak third image B2 the effects are probably negligible. The contribution from the galaxy decreases rapidly towards the blue and in the B band is  $\leq 5\%$  (ref. 4). This small additional flux will be essentially constant for all observations employing an aperture of a few arc seconds. The magnitude differences from the spectrophotometry refer to the continua while the B band photometry includes the C III]  $\lambda$  1,909 line. However the line contributes ≤5% and will be essentially the same for both components and the line strength is not expected to vary9. The third image B2 contributes ≤5% to B1 so its effect will also be negligible<sup>5</sup>.

As for longer term changes the double quasar appears on adjacent POSS plates taken in February 1953 and January 1955. From the O-plates the B mag of component A is 16.9 and  $16.6\pm0.2$  on the two dates respectively, with component B1 = 0.3 mag fainter on both occasions. So both components were -1 mag brighter and the magnitude difference about same as in 1979. From archival plates taken in 1902, 1903 and 1915 Keel<sup>8</sup> finds both components ~0.5 mag brighter than at present and the magnitude difference is  $\approx 0.5$ .

The near constancy of the difference despite an overall 1 mag variation strongly suggests that one component follows the other as would be expected with intrinsic variations and that the time delay is short compared with the intervals between epochs. 0956+57AB can be compared with quasars of similar radio structure as a guide to what optically variability might be expected. Similar objects dominated by an extended steep radio spectrum source<sup>10</sup> undergo slow variations with occasional abrupt changes of slope and periods of constancy. This is seen in Fig. 1. The maximum amplitude that might be expected is 1-1.5 mag (unpublished data) which is well in line with that so far observed.

The photometry does allow some constraint to be placed on difference in light travel times. Long delays are excluded by both the POSS and the 1979 plates showing  $\Delta m = 0.3$ . So  $\Delta t < 24$  yr. From the photometry in Fig. 1, if A follows B1 then  $\Delta t > 1.5$  yr. However, if B1 follows A then  $\Delta t > 2$  yr. This will be increased to ~3 yr if B1 returns to its previous luminosity at the current rate of decline. In a recent analysis Young et al. s gave the time delay  $\Delta t < 6$  yr with component A preceding B1. Combining this with the minimum delay from the discussion above gives a delay  $2 < \Delta t < 6$  yr. If the sense of the delay is correct then the feature recently observed in the light curve of component B1 has already passed in component A and 2 yr at constant luminosity are still to come for B1. The third image B2 is very faint and its effect on the time delays involving B1 will be negligible<sup>5</sup>.

An alternative cause of luminosity variations may be changes within the gravitational lens<sup>11-13</sup>. According to Canizares<sup>14</sup> as an individual star passes through the beam from a quasar the luminosity will be increased typically by several magnitudes. The time scale for this variation is  $\sim 15$  yr but depends on the size of the beam, the mass of the star and the velocities involved. In the case of the double quasar Gott<sup>15</sup> considers that very low mass stars will dominate and produce variations in 1-40 yr. On this basis component B1 is expected to be the more variable as it is seen through regions of higher stellar density than component A at ~5 kpc from the centre of the galaxy. However, Young<sup>16</sup> considered the effects of very low mass stars to be virtually undetectable and suggested that even with a normal stellar population there will be a sufficient number of stars in the beam for the effects to overlap. This means component B1 should vary continuously by up to 50% over 100 yr while component A undergoes the occasional ~15-yr outburst. According to Gott<sup>15</sup> component B1 should generally be brighter than  $I_{B1} = 0.75 I_{A}$  $(\Delta m \le 0.3)$  while Young<sup>16</sup> predicted substantial variation about this value. Both appear to be at odds with the observations for on only one (1980) of the epochs, 1902, 1903, 1915 (Keel)<sup>8</sup>, 1953, 1955 (POSS), 1979, 1980 on which the double quasar has been observed has the ratio been substantially different from  $I_{\rm B} =$  $0.75 I_A (\Delta m = 0.3)$ . With a large beam size the ratio can be kept constant for ~50 yr but it is then impossible to duplicate the rapid changes recently observed. Conversely when the type of variations in Fig. 1 can be modelled the long-term variations are at odds with the prediction. Even in favourable conditions Young<sup>16</sup> considered the recent variations to be too large and too rapid to be due to the passage of stars through the beam. In conclusion the variations in the long and short term are inconsistent with those predicted for the passage of stars through the beam but are consistent with intrinsic variability.

I will make a final comment on the luminosity of the quasars involved in 0957+56AB and the other gravitational lens candidate 1115+080 ABC (ref. 17). If the amplifications of  $\sim 6$ for 0957+56A and ~14 for 1115+08A are correct then the luminosities of the unamplified quasar are  $M_B \sim -23.6$  and -24.4 respectively ( $H = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ , q = 1). These are far in excess of the luminosities of Seyfert galaxies and seriously undermine the suggestion that all quasars are gravitationally lensed Seyfert galaxies<sup>18</sup>

Both components of the double quasar are variable by at least 1.0 mag and show behaviour typical of intrinsically variable quasars with extended radio structure. The near constancy of the difference between the two components at several epochs is contrary to what is expected if the variations are due to the

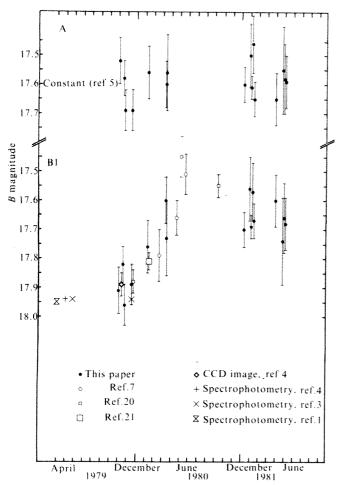


Fig. 1 The light curve of 0957 + 56A and B1 from April 1979 to June 1981. The diagram contains 16 new measurements of both components together with 12 differential measurements from various authors. For the Herstmonceux photometry unfiltered HaO emulsions were used which give magnitudes very close to the B band. Both components were reduced independently relative to a sequence of comparison stars. As no photoelectric sequence is available the relative magnitude scale was obtained through reference to two well calibrated fields from the Herstmonceux quasar monitoring programme <sup>19</sup>. The error in the relative magnitudes is ≤10% and this component is included in the error bars. The zero point of the B magnitude scale is taken as the mean value of component A which is defined as B = 17.6 (as given by Young et al.4) and has an uncertainty of ±0.1 mag. Differential measurements from photometry and spectrophotometry given by other authors are plotted on the assumption that component A is constant throughout this period. This is supported by Keel<sup>8</sup> and by Young et al.5 who find A constant between April 1979 and July 1980.

passage of stars through the beam. Recent photometry places a lower limit of 2 yr on the time delay between the components and if component B1 follows A as calculated by Young et al.5 then the feature observed in the light curve of B1 has already passed in component A. Analysis of archive plates should reveal more about long-term changes but the question of the time delay should be resolved by continued detailed monitoring.

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# First remote sensing of the plasmapause by terrestrial myriametric radiation

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The Earth's plasmapause is a source of natural radio emission in the myriametric wavelength range ( $\lambda_{vac} \sim 10 \text{ km}$ ) and several mechanisms have been proposed for its generation. One such theory advocates the mode conversion of electrostatic waves to electromagnetic radiation by propagation through a radio window. The window properties are such that the escaping terrestrial myriametric radiation is beamed away from the magnetic equatorial plane at an angle which depends on the plasma parameters at the window. This property should allow myriametric radiation observed by spacecraft to be utilized for remote sensing of the plasmapause. An example derived from the satellite GEOS 1 is presented here in which a radial profile of cold plasma density is obtained for the first time by this technique. The method may also allow a better identification of those electrostatic modes existing in the mixture of hot and cold plasma components at the plasmapause and beyond.

Trapped non-thermal continuum (NTC) is used to describe broad-band radio noise at frequencies ≤40 kHz observed by spacecraft in the plasmatrough, the cavity of low-density plasma between the Earth's plasmasphere and magnetosheath1. Escaping NTC is noise at higher frequencies which propagates through the magnetosheath into the solar wind2. NTC is now widely believed to be produced not as a continuum, but as distinct beams of radiation, with different frequencies emanating from different, albeit possibly adjacent regions of the plasmapause<sup>2,3</sup>. After multiple reflections at the plasmatrough boundaries the beams from numerous sources are scattered and merge to produce the radio continuum. Myriametric describes the free space wavelength of the radio noise and brings the nomenclature in line with other planetary beamed emissions<sup>3</sup>.

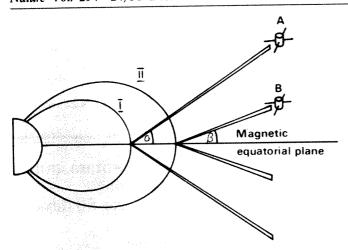


Fig. 1 Representation of the beaming of 30-kHz TMR away from the geomagnetic equatorial plane. The observation of the waves by satellite A allows the position of plasmapause I to be determined. If the observation is made on satellite B, the plasmapause will lie at location II.

The source of terrestrial myriametric radiation (TMR) seems to lie in electrostatic upper-hybrid-resonance (UHR) waves<sup>3</sup> which are observed to be most intense within ±1° of the geomagnetic Equator at and beyond the plasmapause in the morning sector<sup>7</sup>. Theories of TMR production mechanisms have been recently reviewed by Melrose<sup>6</sup>; the one considered here invokes the propagation of UHR wave energy through a radio window<sup>3,4</sup>. The window possesses unique properties in that the TMR will be left-hand polarized and beamed away from the geomagnetic equatorial plane at an angle dependent on the ratio  $f_{ce}/f_p$  at the window, where  $f_{ce}$  and  $f_{pe}$  are the electron cyclotron and plasma frequencies respectively3. These properties allow satellite observations of TMR far removed from the source region to be used to determine the radial profile of cold plasma density at the plasmapause. If the satellite later traverses the source region, in situ measurements should provide confirmation of the derived profile and information on the relationship between hot and cold plasma components at the plasmapause and beyond.

The term 'window' is used here to denote the phenomena whereby waves in one magnetoionic mode can penetrate through a region where, according to simple ray theory, they would be evanescent, and can emerge on the far side in a different magnetoionic mode (see ref. 8 for a recent review of

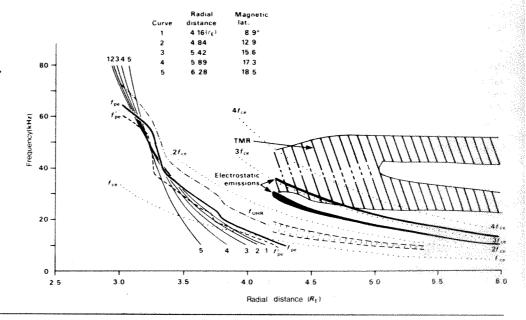
window theory). In the case of propagation of UHR-Z mode waves through a radio window to become left-hand polarized ordinary (L-O) waves, it may be shown that for  $f_{\rm ce} < f_{\rm pe}$ , the 6-dB window half-widths at the plasmapause for TMR frequencies are typically 1°-2°. Such narrow beams, together with the restricted latitudinal dimensions of the UHR source region, allow remote satellite observations of TMR to be utilized to determine the radial position of the window. Thus, because the frequency at which TMR exits from the window is  $f_{pe}$ , the plasma density  $N_e$ , being proportional to  $f_{pe}^2$ , may be determined. Figure 1 illustrates the technique by showing two possible positions, I and II. of the plasmapause. In position I, TMR at 30 kHz is beamed away from the geomagnetic equatorial plane3 at angle  $\alpha = \tan^{-1}(f_{ce}/f_{pe})^{1/2}$ , whereas if the plasmapause is in position II the same frequency would be beamed at a different angle  $\beta$  since  $f_{ce} \propto 1/R_0^3$  has changed,  $R_0$  being the radial distance of the source; the beamwidth in both cases is only 1°-2° and the beams will have mirror images about the equatorial plane. Satellite A, located at radial distance R and magnetic latitude  $\eta$ , will observe TMR source I, whose radial position  $R_0$  may be determined from the equation:

$$R_0^3 R^2 \sin^2 \eta / f_{\text{ceq}} - R_0^2 + 2R_0 R \cos \eta - R^2 \cos^2 \eta = 0$$
 (1)

where  $f_{\rm ceq}$  is the equatorial gyrofrequency at the Earth's surface. A more general expression exists for the case where the satellite and source are not in the same magnetic meridian plane. Similarly, satellite B observations would allow the source position to be determined when the plasmapause is in position II. Note that trapped NTC need not show any beaming effect with respect to the equatorial plane, because after multiple reflections one would expect the plasmatrough to be filled with NTC. However, escaping NTC which has not suffered reflections is synonymous with TMR and should show beaming<sup>2</sup>.

During the period 0200–0400 UT 4 March 1978, a very stable TMR pattern<sup>3</sup> was observed by the satellite GEOS 1 as it moved inbound from  $6.2R_{\rm E}$  to  $4.2R_{\rm E}$ . The satellite changed during that time only 5° long, with respect to a plasmapause source corotating with the Earth. From the way in which the signal intensity increased as the plasmapause was approached it is evident that the source and the satellite were close to being in the same meridian plane. The satellite magnetic latitude decreased nearly linearly from 18° to 8° over the above radial distance. Figure 2 shows a sketch of the TMR spectrogram (see ref. 3 for original) which, beyond  $5R_{\rm E}$  exhibits two bands, one in the region of 50 kHz and the other at lower frequencies; the bands merge into one at smaller radial distances. The variation in the upper and lower frequency limits of the lower band is due to the satellite

Fig. 2 Curves 1-5 show TMR theoretically visible to. GEOS 1 at the five positions shown in the inset table during the inbound leg on 4 March 1978 at ~0600LT. Also shown are the wave emissions seen by the satellite during this period. The hatched region is TMR, whereas the more intense narrow band emissions between gyroharmonics are electrostatic. The weaker emission between  $f_{ce}$  and  $2f_{ce}$  is linked to the cold upper hybrid frequency  $f_{UHR}$ as determined from remote sensing. The 'virtual' and 'real' plasmapause are depicted by  $f'_{pe}$  and  $f_{pe}$  respectively.



intersecting TMR beams of different frequencies during its inbound traverse3. The table in Fig. 2 shows five satellite locations at which equation (1) was applied assuming, in the first instance, that the whole frequency range 10-80 kHz was visible to GEOS 1. The corresponding source profiles are labelled 1-5 in Fig. 2, and it is immediately apparent that the five curves all intersect close to  $50 \, \text{kHz}$  at  $3.22 R_{\text{E}}$ . The significance of this is that if the plasma frequenc is  $50 \, \text{kHz}$  at  $3.22 R_{\text{E}}$ , the satellite should observe that frequency during the whole of its inward traverse from  $6.2R_{\rm E}$  to  $4.2R_{\rm E}$ , which indeed it does

By utilizing the spectrogram and a complete family of theoretical curves similar to 1-5 in Fig. 2, one can determine the source positions at other frequencies and hence obtain a radial profile of plasma density. For example, if at a given frequency and satellite position no TMR is observed, this indicates that the source does not lie on the corresponding theoretical profile, whereas if TMR is visible the source position for that frequency can be determined from the relevant curve. The radial plasma frequency profile constructed in this way for 4 March 1978 is labelled  $f'_{pe}$  in Fig. 2. A small correction to allow for wave refraction at the window has to be applied to this profile as equation (1) was derived assuming linear propagation. Ray tracing calculations indicate that  $\sim 0.08R_{\rm E}$  has to be added to the radial positions of the sources and thus the 'true' plasma frequency profile is shown labelled  $f_{pe}$ .

Figure 2 shows (dotted) the electron cyclotron frequency  $f_{ce}$ and its harmonics. The strongest natural emission band observed in the spectrogram lies between  $2f_{ce}$  and  $3f_{ce}$ , whereas the much weaker emission between  $f_{ce}$  and  $2f_{ce}$  is the one that links smoothly to the  $f_{\rm UHR}$  profile as calculated from  $f_{\rm UHR} = (f_{\rm pe}^2 + f_{\rm ce}^2)^{1/2}$ . Note that GEOS 1 was at magnetic latitudes greater than 8° when the spectrogram was recorded. Unfortunately, the satellite was subject to its end of orbit switch-off before it traversed the predicted source region and hence no direct in situ observations could be made on that particular pass. However, on the previous day, GEOS 1 remained in operation as the equatorial plane was crossed and the TMR observations, although themselves more limited in extent and more complicated in spectrum than on 4 March indicated that the plasmapause position was in approximately the same position. As for 4 March 1978, the strongest electrostatic emissions lay between  $2f_{ce}$  and  $3f_{ce}$  when the satellite was at magnetic latitudes  $\geq 3^{\circ}$ , but as the Equator was approached the weak emission between  $f_{ce}$  and  $2f_{ce}$  increased in intensity and became the most intense at the Equator. Therefore, from the indirect evidence on 4 March and the more direct evidence on 3 March, the cold UHR emission apparently dominates only very close to the Equator, whereas the stronger emission at other latitudes is at a higher frequency. A detailed study of this effect and of the possible extension of this technique to separate hot and cold plasma components will be reported separately.

The main conclusions, therefore, are that the unique properties of the radio window from which TMR appears to emanate can be used for remote sensing of the plasmapause. When the technique is applied to certain GEOS 1 data, the results indicate that the stronger non-equatorial electrostatic emissions are at frequencies higher than the cold UHR frequency. Emissions at the latter frequency dominate only within ~±1° of the geomagnetic Equator, and their identification may allow the separation of hot and cold plasma populations. As similar emissions to TMR and to NTC have been observed by the Voyager spacecraft at Jupiter and Saturn, these results could have considerable significance for the understanding of the environments of other planets, and could provide a diagnostic tool to study regions of other planetary magnetospheres not accessible to in situ observations by spacecraft.

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# Atmospheric angular momentum and the length of day: a common fluctuation with a period near 50 days

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Semiannual, annual and some longer period changes in the rotation of the Earth have long been attributed to meteorological causes<sup>1-3</sup>. More recently some higher frequency variations in the length of day (l.o.d.) were shown to be strongly correlated with variations of the polar component of the angular momentum of the atmosphere<sup>4,5</sup>. However, fluctuations in l.o.d. with periods near 50 days were not definitely identified, because either the sampling was too coarse4 or it extended over too short an interval<sup>5</sup>. Fluctuations in tropical wind speeds with periods in this range have, nevertheless, been studied<sup>4,6,7</sup>, but their counterparts, although expected4, had not been identified in the Earth's rotation. Last year, four astronomical measures of changes in l.o.d. obtained in 1979 were shown to exhibit nearly the same ~50-day fluctuation8. To find out whether this fluctuation was persistent and of meteorological origin we analysed lunar laser ranging (LLR) observations from the McDonald Observatory in Texas and zonal (east-west) wind data deduced from sources distributed over the globe. The relevant data were available for the common period 1976-79. We describe these analyses in turn and then compare the results.

Values of l.o.d. were obtained by smoothing and then differentiating our LLR estimates of the rotation of the Earth as a function of time. These values were corrected for the effects of polar motion with pole positions determined by the Bureau International de l'Heure<sup>10</sup>. Our results were tabulated at 5-day intervals from 2 January 1976 to 27 December 1979. Typically each tabulated value of l.o.d. has an estimated standard error of ~0.1 ms. The uncertainties, however, vary over the 4-yr interval, due in part to the uneven distribution in time of the LLR observations.

To investigate the short-term variations in l.o.d., we used an ad hoc procedure that seems adequate for the purpose. First we divided the l.o.d. time series into nearly year-long segments. For each segment we determined the mean level and, using least squares analysis, the best-fitting Fourier components with annual and semiannual periods. We then removed these components, as well as the fortnightly and monthly tidal terms, from the l.o.d. values. The residual variations are shown for each year in Fig. 1a-d. Each curve is dominated by fluctuations with periods in the range 40-60 days. The typical amplitude of these fluctuations is  $\sim 0.2$  ms, or about half the mean amplitudes of the annual and semiannual terms; the lack of continuity between the curves is due to the procedure used to remove the low-frequency components of the l.o.d. variations.

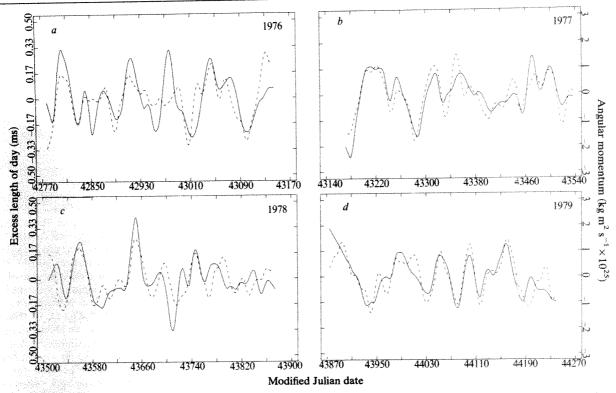


Fig. 1 Variation in length of day determined from lunar laser ranging observations (solid line) and from the angular momentum of the atmosphere (dashed line) for the years 1976-79. The annual and semiannual variations and mean levels of both sets of l.o.d. values, as well as the fortnightly and monthly tidal terms in the lunar laser ranging values, have been removed (see text).

Although appropriate data on the angular momentum of the atmosphere are not available for the time before 1976, useful lunar laser ranging observations extend back to 1971. These, too, exhibit significant fluctuations with periods in the range 40-60 days. Power spectra of the entire set of l.o.d. values, and subsets of them, will be presented and discussed elsewhere.

Our time series of values of the angular momentum of the atmosphere was derived from analyses of the global distribution of zonal winds made twice daily (for 00.00 and 12.00 UTC) by the US National Meteorological Center (NMC)<sup>11</sup>. These analyses are based on data gathered from various sources, including rawinsonde balloons, satellites and commercial aircraft, and provide wind velocities over a grid with points spaced every  $2.5^{\circ}$  in both latitude and longitude, at each of 12 pressure levels ranging from 1,000 to 50 mbar. (A detailed analysis of the spatial and temporal variability of these zonal winds will be presented elsewhere 12.) The values of wind velocity at the grid points were zonally averaged and then, with appropriate weighting, integrated over latitude ( $\phi$ ) and pressure (p) to determine, relative to an Earth-fixed frame, the approximate angular momentum of the atmosphere about the polar axis:

$$h_3(t) = \frac{2\pi a^3}{g} \int_{100 \text{ mbar}}^{1,000 \text{ mbar}} \int_{-\pi/2}^{\pi/2} [u] \cos^2 \phi \, d\phi \, dp$$
 (1)

where [u] is the zonal average of the eastward component of wind velocity, a is the mean radius of the Earth and g is the acceleration due to gravity. Equation (1) is based on the assumption that the atmosphere is in hydrostatic equilibrium and ignores the variation with altitude and latitude of the distance of a parcel of air from the centre of the Earth. Because of the expected unreliability of the data at pressures below 100 mbar (ref. 13), we have ignored these data. Test calculations, using the low pressure data, indicate that deleting them incurs a systematic error of  $\sim 10\%$  in the determination of the mean value of  $h_3$ , but we expect this deletion to have an even smaller impact on day-to-day changes in  $h_3$ . Of greater effect on these changes are the errors that result from gaps in the spatial

distribution of observations, inaccuracies in the basic wind measurements and approximations in the NMC's analysis. We cannot place a reliable quantitative limit on these errors; however, model calculations related to the first two error sources  $^{14}$  and comparisons related to the third indicate that these errors in toto are typically  $\sim 10^{20}$ % or less.

We determined  $h_3$  from equation (1) for twice-daily epochs daily extending from 1 January 1976 to 31 December 1979. The results from December 1978 to November 1979 are thought to be the most accurate because of the intense effort devoted to data collection under the Global Weather Experiment<sup>15</sup>.

Fluctuations in  $h_3$  on time scales of the order of 1 yr or less probably affect only the crust and mantle (hereafter 'shell') of the Earth because the core is thought to participate only in longer period variations<sup>5,16</sup>. For variations with periods <1 yr, then, the relation between the changes  $\Delta h_3$  in  $h_3$  and the corresponding changes  $\Delta \omega$  in the angular velocity of the shell of the Earth is given by

$$\Delta\omega \cong -\frac{\Delta h_3}{I_s} \tag{2}$$

where  $I_s$  is the principal (axial) moment of inertia of the shell. A change in the angular velocity of the shell is related to a change in the observed l.o.d. by

$$\frac{\Delta l.o.d.}{l.o.d.} = -\frac{\Delta \omega}{\omega} \tag{3}$$

Combining equations (2) and (3) and using the following constants:  $I_s \approx 7.04 \times 10^{37} \text{ kg m}^2$  (this value of  $I_s$  was derived from the B1 density model in ref. 17; the uncertainty in  $I_s$  is probably not greater than 2%),  $\omega \approx 7.29 \times 10^{-5} \, s^{-1}$ , l.o.d. = 86,400 s, yields

$$\Delta l.o.d.(s) = 1.68 \times 10^{-29} \, \Delta h_3 \, (kg \, m^2 \, s^{-1})$$
 (4)

The mean level of  $h_3$  between 1 January 1976 and 31 December 1979 is  $\sim 1.4 \times 10^{26}$  kg m<sup>2</sup> s<sup>-1</sup>. This amount of angular momentum transferred to the solid Earth (excluding its core) would change the l.o.d. by 2.3 ms.

As with the values derived from LLR observations, we removed from the l.o.d. values obtained from equations (1) to (4) the mean level and the best-fitting Fourier components with annual and semiannual periods for each yr-long segment separately. The residuals were smoothed using a gaussianshaped low-pass filter, with a window width in the time domain of about 8 days (full-width-at-half-maximum). This degree of smoothing is consistent with the smoothing applied to the LLR estimates of Earth rotation. Figure 1a-d shows the smoothed curves for each year superimposed on the l.o.d. curves obtained from the lunar laser ranging observations. In common with the LLR curves, those derived from the estimates of  $h_3$  are discontinuous from year to year. The scale on the right-hand side is related to the scale on the left through equation (4).

The degree of correlation between the observed (LLR) and the inferred (meteorological) values of the changes in l.o.d. is quite striking; in most places there is good agreement in both the amplitude and phase of the variations. The correlation seems highest in 1979, perhaps due at least in part to the special attention devoted in that year to the collection of meteorological data. Overall, the comparison in Fig. 1 implies strongly that these ~50-day period fluctuations in l.o.d. are 'real' and are related to meteorological effects. The uncertainties in the LLR l.o.d. values and in the estimates of  $h_3$  are large enough to account for the differences in the two sets of curves and therefore too large to make a detailed analysis of these differences worthwhile. These uncertainties are also too large to permit us to place useful limits on the magnitudes of any non-atmospheric contributions to the changes in l.o.d. Thus, we cannot determine from the comparison whether the core participates in these l.o.d. changes nor can we place useful constraints on the contributions of ocean currents and other neglected torques. Other studies 16 imply that the contributions of ocean currents are small. Our calculations indicate that the contributions of other neglected torques are also small.

The exchange of momentum between the Earth's atmosphere and surface has a central role in determining both the structure of atmospheric flow and the dynamics of the Earth's rotation. Observed changes in l.o.d. can provide a constraint for models of atmospheric flow and a (partial) check for global analyses of such motions. The removal of more accurate estimates of the atmospheric contribution to changes in l.o.d. observations might reveal residual variations associated with ocean currents, earthquake fault displacements and core-mantle interactions.

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# Solvated electrons in the upper atmosphere

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Solvated electrons have been postulated 1-3 to exist in liquids and solids, and recent laboratory measurements4 have revealed that they exist even in the gas phase. In particular, these measurements suggest that eight water molecules are sufficient to bind an electron and give rise to a solvated electron of the kind (H<sub>2</sub>O)<sub>8</sub>. This seems to be consistent with theoretical considerations<sup>5</sup> suggesting that a dipole of molecular size must have a minimum dipole moment of about 2.5 D to support a bound state. According to theoretical calculations<sup>6</sup>, a cold, neutral water cluster (H<sub>2</sub>O)<sub>8</sub> has a dipole moment of 4.61 D, so that larger (H<sub>2</sub>O), clusters may be expected to bind an electron. Building on these ideas I consider here the possibility that solvated electrons may also exist in the Earth's upper atmosphere and that they may affect nucleation and aerosol formation. Furthermore, I suggest that upper-atmosphere solvated electrons may be detected, using rocket-borne ion mass spectrometers, and that they may be used as tracers for probing condensation nuclei.

In the Earth's upper atmosphere solvated electrons may be formed by attachment of free electrons to clusters of molecules having large dipole moments or polarizabilities. Thus, solvated electron formation would be restricted to regions where both free electrons and the corresponding neutral species are present.

In the atmosphere free electrons essentially exist only at heights above  $\sim 60-65$  km (day) and 75-80 km (night), whereas negative ions represent the atmospheric negatively charged constituents below these heights<sup>7</sup>. The main reason for the rather abrupt transition from a negative ion to an electron regime is electron detachment induced by oxygen atoms<sup>8</sup> which become abundant above these heights'.

Collisions of free electrons with these molecular clusters may give rise to solvated electrons.

The pHs should, in most conditions, not be large enough to give rise to solvated electrons. Sufficiently large pHs and  $(H_2O)_n$ clusters probably occur only in conditions of extremely low temperatures (<150 K) around the mesopause<sup>9</sup>. Such conditions are occasionally met at high geographical latitudes during local summer. In fact, large pHs containing up to 20 water molecules were recently detected by Björn and Arnold 10 using a

rocket-borne ion mass spectrometer.

It has been suggested previously<sup>9,11</sup> that pHs may induce the nucleation of atmospheric water vapour in the cold mesopause region and thereby give rise to a mesospheric haze layer and to noctilucent clouds. As pointed out elsewhere, however, ion nucleation, although being thermodynamically possible, may suffer from severe kinetic limitations. pHs may be rapidly destroyed by dissociative electron recombination and therefore not grow to critical sizes.

Here, I propose an alternative recombination mechanism involving solvated electron formation. Instead of neutralizing the excess proton contained in a large pH the electron may be solvated by water molecules attached to the proton. Thus, a charge doublet composed of a solvated electron and a solvated proton may be formed. Taking measured enthalpy differences for successive proton hydration12 it seems that 11 water molecules are sufficient to stabilize a pH against spontaneous neutralization of its excess proton. In fact, this number of H<sub>2</sub>O molecules is already larger than the eight required for electron solvation.

A doublet may grow by attachment of water molecules and ultimately pick up another electron and thereby be converted to a triplet containing a solvated proton and two solvated electrons. The formation of positive triplets is much less likely as the rate of positive ion collisions is much less than that of electron collisions due to the about 100,000 times larger ion mass. As the triplet carries a negative net charge, it should readily recombine with a positive ion, generally a pH giving rise to the formation of a quasineutral quadruplet. Further electron and ion attachment may lead to larger multiplets. These resemble the stratospheric polyions or multi-ion complexes I recently discussed<sup>13</sup> which, however, contain negative ions rather than solvated electrons.

If the charge multiplets were in fact formed at the mesopause, they may have an important bearing on nucleation, as they may stabilize subcritical water cluster against evaporation and allow for the build-up of critical clusters by electron, pH, and H<sub>2</sub>O attachment.

I now attempt to estimate the abundances of doublets and quadruplets at the mesopause for a steady state situation.

Assuming that doublets are formed by electron recombination of large pHs (fractional abundance f) at a rate coefficient a and are lost only by electron attachment (rate coefficient k), the following steady-state continuity equation is obtained

$$f[pH]\alpha_{e}[e] = [D]k[e]$$
 (2)

which relates the concentration of doublets [D] to the pH and total electron concentrations. Taking  $\alpha_e = 10^{-5} \text{ cm}^3 \text{ s}^{-1}$  (ref. 14) and assuming that every collision of an electron and a doublet leads to electron attachment  $(k = 10^{-7} \text{ cm}^3 \text{ s}^{-1})$  we obtain

$$[\mathbf{D}] \approx f \mathbf{1} \mathbf{O}^2[p\mathbf{H}] \tag{3}$$

Analogously we obtain the steady-state continuity equation for triplets

$$[\mathbf{D}]k[\mathbf{e}] = [\mathbf{T}]\alpha_{\mathbf{I}}[\mathbf{n}_{+}] \tag{4}$$

where  $\alpha_{I}$  is an ion-ion recombination coefficient (usually 15,16  $\sim 6 \times 10^{-8} \text{ cm}^3 \text{ s}^{-1}$ ) and  $[n_+]$  is the total positive ion concentration. Assuming  $[n_+] = [pH] = [e]$  we obtain

$$[T] \approx [D] \tag{5}$$

Evidently the above estimates can, if at all, only apply for f smaller than 0.01, because otherwise triplets would become more abundant than electrons. This would give rise to a depletion of electrons by triplet formation which is in conflict with the assumption of  $[e] = [n_+]$ . Hence, if f was >0.01, one should expect that some depletion of electrons actually occurs, the rates for doublet and triplet formation, however, decrease.

Note that a decrease of [e] due to electron solvation would increase the lifetime of smaller pHs, which are destroyed by dissociative electron recombination, and thus would increase the rate of formation of large pHs. Thus, there would be a strong positive feedback in ion nucleation.

Electron solvation may, of course, be induced by  $(H_2O)_n$ clusters also if these are not formed by ion nucleation but by some other process, as for example, homogeneous nucleation of water vapour condensation on pre-existing particles.

Having discussed electron solvation by neutral and positively charged water clusters, I now consider electron solvation by 'meteoric smoke particles'. These molecular clusters may contain compounds with large dipole moments and polarizabilities which enable them to solvate an electron strongly, because the presence of meteor smoke particles is probably not limited in space and time electron solvations by these clusters may be a common process around the mesopause.

Taking surface area densities for meteoric smoke particles predicted by the model of Hunten et al.<sup>17</sup> and assuming that solvated electrons are lost by recombination with positive ions, an upper limit to the abundance ratio for solvated electrons and electrons of about 0.1 is estimated.

Finally, if solvated electrons were indeed as abundant as estimated above, they should be easily detectable in the meso-

pause region using rocket-borne mass spectrometers. In situ mass spectrometric detection of solvated electrons may be a powerful tool of probing molecular clusters such as (H<sub>2</sub>O)<sub>n</sub> and meteoric smoke particles in the mesopause region.

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# Halocarbons in the stratosphere

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The possible impact of chlorine compounds on the Earth's ozone layer has caused concern. Profiles of the anthropogenic halocarbons F-11 (CFCl<sub>3</sub>) and F-12 (CF<sub>2</sub>Cl<sub>2</sub>) have already been measured in the stratosphere1-4. Measurements of the vertical distribution of methyl chloride (CH3Cl), the most important natural chlorine-bearing species confirm that chlorine of anthropogenic origin now predominates the stratosphere<sup>5</sup> More halogen radicals are added through decomposition of various other halocarbons, most of them released by man. We report here the first measurements of vertical profiles of F-13 (CF<sub>3</sub>Cl), F-14 (CF<sub>4</sub>), F-113 (C<sub>2</sub>F<sub>3</sub>Cl<sub>3</sub>), F-114 (C<sub>2</sub>F<sub>4</sub>Cl<sub>2</sub>), F-115 (C<sub>2</sub>F<sub>5</sub>Cl), F-116 (C<sub>2</sub>F<sub>6</sub>), and F-13 B(CF<sub>3</sub>Br) resulting from gas chromatography-mass spectrometer (GC-MS) analysis of air samples collected cryogenically between 10 and 33 km, at 44° N. Some data for F-22 (CHF<sub>2</sub>Cl), methyl bromide (CH<sub>3</sub>Br) and methyl chloroform (CH<sub>3</sub>CCl<sub>3</sub>) also presented are subject to confirmation.

Cryogenic sampling with subsequent analysis of the samples in the laboratory is an excellent tool for simultaneously measuring a variety of stable trace gases in the atmosphere 1,2; vertical profiles of H<sub>2</sub>, CH<sub>4</sub>, CO, CO<sub>2</sub>, N<sub>2</sub>O, CFCl<sub>3</sub>, CF<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>Cl have already been measured up to ~36 km altitude. From a recent balloon flight of the MPAE neon-cooled sampler launched on 25 September 1980, in southern France (44° N), seven stratospheric air samples were recovered. While the first one of these had been taken during the ascent at 10 km height, the six other samples were collected during the descent of the heliumfilled balloon at 33.2, 28.8, 25.9, 23.3, 20.0 and 14.4 km, respectively. These figures correspond to the centres of the sampling height intervals whose ranges varied between 0.2 and 0.5 km. The slow descent, at a rate of ~50-100 m min<sup>-1</sup>, was achieved be releasing gas through a remotely controlled valve on top of the balloon.

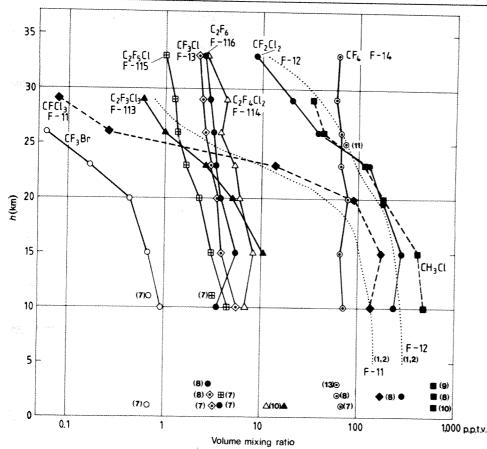


Fig. 1 Vertical profiles of halocarbons measured at 44° N by GC-MS analysis of cryogenically collected balloon samples. Results from measurements made by other authors (reference numbers in parentheses are shown for comparison). The tropospheric data shown at the bottom are not related to the height scale.

Immediately after the flight, the samples were transferred into 1.6-l stainless steel bottles electropolished internally and fitted with metal bellows valves. Each bottle contained a sample of about 8-101 of air at STP that was analysed three weeks later using a GC-MS combination at AERE Harwell (Hewlett Packard 5710 GC/VG Micromass 16 FMS). As repetitive analyses of previously collected samples had shown, F-13, F-14, F-113, F-114, F-115, F-116, and F-13B remain stable in these bottles over a period of more than 6 months (S.A.P. and R. A. Rasmussen, unpublished data). Because many of the concentrations to be measured were below the detection limit for direct analysis of a 5-ml sample, a cryotrap concentrator described elsewhere was used for all analyses. The volumes of air used for each estimation varied between 100 ml and 1 l, which is a substantial fraction of the total sample. Duplicate analyses were generally not performed so that data on a wide range of species could be recorded. The precision of the analysis is  $\pm 15\%$  for every individual data point, while the error of the absolute calibration is  $\pm 10\%$  for all constituents shown here. Typically the detection limit is of the order of 0.1 p.p.t.v. (1 p.p.t.v. = 1 part in 1012 by volume) for a 1-l sample.

The results are plotted in Fig. 1. The upper parts of some of the profiles, where the mixing ratio fell below the detection limit, are not shown. Measurements made by other authors are included for comparison.

The low-altitude sample collected during the ascent at 10 km was obviously diluted by the helium that had been filled into the air intake system of the cryogenic sampler before launch. This is confirmed by the comparison of the data from the 10-km balloon sample with those obtained from samples collected cryogenically aboard a Learjet aircraft over southwestern UK at almost the same time and altitude (S.A.P. and R. A. Rasmussen, unpublished data). For most constituents, the mixing ratios analysed from the balloon sample taken at 10 km are lower than those obtained from the Learjet samples. A correction factor can be applied to this lowest balloon sample based on measurements of common constituents such as F-11 and F-12 in both balloon and aircraft samples. This suggests that the mixing

ratios in the 10-km balloon sample are  $\sim$ 20% low, which needs to be born in mind when referring to the profiles shown in Fig. 1.

Stratospheric profiles of CFCl<sub>3</sub>, CF<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>Cl have already been documented<sup>1-6</sup>. Below ~20 km the mixing ratios of both CFCl<sub>3</sub> and CF<sub>2</sub>Cl<sub>2</sub> are higher than those given by the average profiles measured in June 1979 at the same latitude<sup>1.2</sup>, also shown in Fig. 1 (dotted lines). Between June 1977 and June 1979, an increase of the average tropospheric mixing ratio of CFCl<sub>3</sub> from 130 to 147 p.p.t.v. had been observed at 44° N. During the same time CF<sub>2</sub>Cl<sub>2</sub> increased from 240 to 292 p.p.t.v. (refs 1, 2). The increase between June 1979 and September 1980 shown here is in reasonable agreement with that observed earlier. Above 25 km the mixing ratio of F-11 falls off more rapidly than the average profile for 1979.

The stratospheric profile obtained for CH<sub>3</sub>Cl agrees fairly well with that measured in June 1978 <sup>5</sup> if the uppermost point is omitted. The increase of the CH<sub>3</sub>Cl mixing ratio above 30 km may be due to contamination of the sample collected at ceiling altitude. Three near-surface measurements of 630 p.p.t.v. (ref. 8), 624 p.p.t.v. (ref. 9) and 611 p.p.t.v. (ref. 10) are shown at the bottom of Fig. 1 for comparison. Their display, which was made for the sake of clarity, has no relation to the height scale of Fig. 1. The same holds for the other tropospheric data plotted in Fig. 1. CH<sub>3</sub>Cl is a natural constituent with the ocean being its main source<sup>9</sup>.

The other halocarbons whose vertical distributions are shown in Fig. 1 have, to our knowledge, not yet been measured in the stratosphere, with the exception of F-14 ( $CF_4$ ) for which one data point—75 p.p.t.v. at 25 km—was measured by Goldman et al. 11. In the troposphere some measurements exist for most of these constituents, and these data have been included at the bottom of Fig. 1 to be identified by the same symbols as used in the stratospheric profiles. Two data points for  $CF_3Br$  and  $C_2F_5Cl$ , respectively, as analysed from aircraft samples taken at 11 km (ref. 7) are also shown for comparison.

F-13 B (CF<sub>3</sub>Br), a bromine compound of anthropogenic origin, is used as a fire extinguisher. Its abundance, about 0.7 p.t.t.v. in the troposphere<sup>7</sup>, is mostly due to anthropogenic

release. In the stratosphere CF<sub>3</sub>Br mixing ratios decrease, from about 1 p.p.t.v. at 10 km to 0.06 p.p.t.v. at 25.9 km. It has been argued7 that this decrease may be due to photolysis by which bromine is released augmenting the effect that natural bromine has on removing ozone. Methyl bromide (CH<sub>3</sub>Br) is, like methyl chloride, predominantly of a natural origin. Tropospheric measurements of CH3Br suggest mixing ratios ranging between 5 and 25 p.p.t.v. (ref. 8). If our measurements are correct, then in the stratosphere the mixing ratio of CH<sub>3</sub>Br falls off rapidly: 1.2 p.p.t.v. was measured at 14.4 km, while the sample taken at 20 km was already below the detection limit. These data are not shown in Fig. 1.

The aluminium industry<sup>7</sup> is probably the major source for both F-14 (CF<sub>4</sub>) and F-116 (C<sub>2</sub>F<sub>6</sub>). F-14 is a very stable constituent12 showing almost no vertical gradient in the stratosphere. Its mixing ratio was measured as 65 p.p.t.v. at 14.4 km and 62 p.p.t.v. at 33.2 km; the single data point measured by Goldman et al.11 fits this profile quite well. Tropospheric CF4 mixing ratios, with averages of 69.9 p.p.t.v. (ref. 7), 63.6 p.p.t.v. (ref. 13) and 65 p.p.t.v. (ref. 8) measured in the Northern Hemisphere, match our stratospheric data within the quoted error limits. F-116 decreases from about 4 p.p.t.v. measured at 10 km to 2.5 p.p.t.v. at 33.2. This difference from F-14 may be due to a more rapid buildup in the atmosphere or due to stratospheric decomposition. The tropospheric mixing ratio of 4 p.p.t.v. measured in 1979 is in good agreement with our data.

The results for F-13 (CF<sub>3</sub>Cl) and F-115 (C<sub>2</sub>F<sub>5</sub>Cl) are interesting. It was suggested earlier that F-115 is growing more rapidly than other anthropogenic species containing the CF<sub>3</sub> grouping, possibly because it is used in conjunction with F-22 (CHF<sub>2</sub>Cl) in the refrigerant F-502. This may account for the steeper profile for F-115 whose mixing ratios decrease from 3.1 p.p.t.v. at 14.4 km to 1.0 p.p.t.v. at 33.2 km, compared with that for F-13 which falls from 3.9 p.p.t.v. at 14.4 km to 2.3 p.p.t.v. at 33.2 km. A comparison with tropospheric F-13 mixing ratios, of 3.4 p.p.t.v. (ref. 7) and 3.0 p.p.t.v. (ref. 8) measured in 1979, seems to suggest that F-13 is also increasing with time. For F-115 a tropospheric mixing ratio of 4.1 p.p.t.v. was measured in 1980 (ref. 7).

Our stratospheric profile for F-22 shows a decrease from  $\sim 50$ p.p.t.v. at 10 km to ~10 p.p.t.v. at 33.2 km. This is an important species to measure both because of its rapid growth rate14 and because it contains a hydrogen atom and therefore will react with hydroxyl radicals in the stratosphere. Our data must be considered preliminary though, because of inconsistencies apparent on reanalysis after a 3 months gap. The profile is therefore not shown on Fig. 1.

F-113 (C<sub>2</sub>F<sub>3</sub>Cl<sub>3</sub>) and F-114 (C<sub>2</sub>F<sub>4</sub>Cl<sub>2</sub>) are used as refrigerants. Their tropospheric abundances, which have been measured extensively by Singh et al.10, are 19 and 12 p.p.t.v., respectively, on the Northern Hemisphere. In the stratosphere F-113 is rapidly decomposed, its mixing ratio decreases to 0.6 p.p.t.v. at 28.8 km. The stratospheric decomposition of F-114 is much slower; its mixing ratios decrease from about 8.5 p.p.t.v. at 14.4 km to 2.7 p.p.t.v. at 33.2 km.

Methyl chloroform (CH<sub>3</sub>CCl<sub>3</sub>), mainly used as a dry cleaning solvent, has been suggested 10 as a potential depleter of stratospheric ozone due to its rapid growth in the lower atmosphere (15.5 p.p.t.v. yr<sup>-1</sup>). For the Northern Hemisphere Singh et al.<sup>10</sup> published an average mixing ratio of 113 p.p.t.v., based on measurements made at the end of 1977, while Rasmussen and Khalil<sup>8</sup> measured an average of 135 p.p.t.v. during mid-1979. Our vertical profile measured in the stratosphere indicates that CH<sub>3</sub>CCl<sub>3</sub> is rapidly decomposed. The mixing ratios decrease from  $\sim 100$  p.p.t.v. at 10 km to 1 p.p.t.v. at 23.3 km. These measurements were made 3 weeks after the samples had been taken. Analyses taken 3 months later showed that methyl chloroform had completely disappeared from the containers (carbon tetrachloride had already disappeared 3 weeks after sampling, when the first analyses were made; thus no CCl<sub>4</sub> profile can be presented). Because the above values for CH<sub>3</sub>CCl<sub>3</sub> are preliminary they are not displayed in Fig. 1. As the

present annual growth rate of CH<sub>3</sub>CCl<sub>3</sub> is much faster than that of CFCl<sub>3</sub> (ref. 10) it might add comparable amounts of ClO<sub>x</sub> radicals to the stratosphere within a forseeable time.

Our results demonstrate that besides F-11, F-12 and CH<sub>3</sub>Cl there are quite a few halocarbons, mostly anthropogenic, that contribute to the stratospheric halogen budget. Above 30 km the amount of organically bound chlorine present as F-113, F-115, F-13, F-114, and F-22 combined is comparable with that present as F-11 and F-12.

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### Anomalous wind estimates from the Seasat scatterometer

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The Seasat-A Satellite Scatterometer(SASS) measured the radar backscatter intensity from the sea surface using a fourbeam microwave antenna<sup>1-3</sup>. Estimates of wind speed and direction derived from these data agree well with surface measurements made during the Joint Air-Sea Interaction (JASIN) experiment<sup>4-6</sup>, but there are occasions (3 out of 23 satellite passes) when the results are anomalous. We have now studied one such occasion when the satellite measurements differed substantially from those at the surface of the sea and conclude that the interpretation of the SASS measurements may have been vitiated by a mid-level convective system deep enough to produce thunderstorms and lightning.

Figure 1 shows a plot of the SASS wind vectors for Rev. 557 (23.16 GMT, 4 August 1978) together with wind speeds measured by ships at the corners of the JASIN meteorological triangle. Over most of the area SASS winds are 7 m s<sup>-1</sup> or less but in a region centred on 59.1 °N and 12.0 °W, the SASS speeds are about 17 m s<sup>-1</sup>, which, given the rather weak pressure gradient, seems unlikely. Moreover, examination of a similar SASS plot for Rev. 556, 100 min earlier, revealed no apparent irregularities at the same location.

To understand this discrepancy we examined the individual normalized radar backscatter coefficients,  $\sigma^{\circ}$ —the basic scatterometer data from which wind vectors are derived. The region of anomalously high winds exhibited localized, large  $\sigma^{\circ}$  obtained

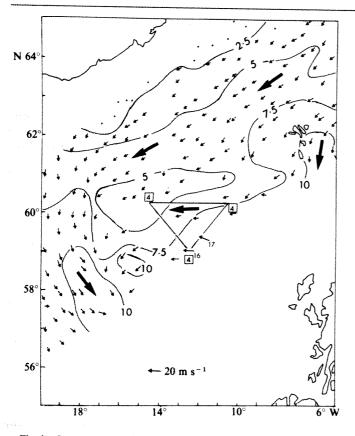


Fig. 1 Scatterometer wind vectors for Rev. 557, 23.16 GMT on 4 August 1978 (wind speeds in m s<sup>-1</sup>). The harmonic nature of the algorithm used to convert backscatter intensity to wind velocity means that up to four solutions are obtained for each footprint<sup>10</sup>, each having similar speeds but differing widely in direction. A preferred direction has been manually selected in each case to ensure dynamic consistency and to give closest agreement with routinely produced surface pressure fields. Anomalous speeds near the southern corner of the JASIN meteorological triangle have been annotated and points without arrows indicate wind speeds of 3 m s<sup>-1</sup> or less. Numbers in boxes denote speeds measured by ships at the corners of the triangle.

from the backward and forward looking antennas (beams 3 and 4 respectively) on the left-hand side of the satellite for both vertical and horizontal polarizations. Figure 2 shows contour plots of  $\sigma^{\circ}$ , adjusted to remove incidence angle dependence, for beam 3 vertical polarization. High-backscatter regions along a 300-400 km line lying to the south-east of the JASIN meteorological triangle and aligned NE/SW were not present on the previous pass although near 59 °N 10.5 °W there was a suggestion of increased  $\sigma^{\circ}$ . This corresponded closely with a region of increased liquid water as inferred from the Scanning Multichannel Microwave Radiometer (SMMR) (Fig. 3) and also with a small feature in the synthetic aperture radar image apparently oriented ESE/WNW. Unfortunately, the sampling characteristics of these two instruments meant no information in the area of interest could be obtained on Rev. 557. As these results suggested that the scatterometer anomalies were associated with a meteorological event of rather small scale, the meteorological data available for the JASIN area were studied.

The UK Meteorological Office analysis at 18.00 GMT showed fronts approaching the area from both the west (front A) and from the north-east (front B). Time series of surface winds from several platforms showed that at the passage of B the wind veered by 50° and increased by 3-4 m s<sup>-1</sup>. The times at which this change occurred at each of the platforms suggested southwestward propagation of the front at 5 m s<sup>-1</sup>. A NOAA-5 IR satellite image at 18.16 GMT revealed that B was not accompanied by a well-defined band of cloud. However, at 58.5 °N, 9.5 °W there was evidence of deep convective cloud. Subsequent cloud pictures at 20.09 and 21.33 GMT from the Seasat visible

and IR radiometer (VIRR) indicated that the feature moved westwards at  $\sim$ 7 m s<sup>-1</sup> to occupy the area 58-59 °N and 10-12 °W at 21.33 GMT which is close to the position of the liquid water maximum observed by the microwave radiometer at the same time. The shape of the cloud mass resembles that of the region of high backscatter at 23.16 GMT.

Some idea of the structure of the upper air in between fronts A and B can be gained from the JASIN radiosonde ascents. On 4 August ascents were made at approximately hourly intervals throughout daylight hours from ships at the corners of the 200-km triangle. Preliminary analysis indicates similar structure at all three corners. The most significant feature is the deep, moist layer in middle levels of the troposphere. At the base of this layer there is a marked temperature inversion such that convection from the surface cannot penetrate above a height of ~2 km. However, the air above would be quite unstable if lifted by a small amount and could support deep convection extending from 2 to 6 km. The inversion marks the frontal surface of a cold occlusion and it is when the surface front passes that the wind veer occurs. Convergence at this feature could release the potential instability aloft.

Upper-air winds were deduced from Loran-C tracking of the sondes and, assuming the convective system to be steered with the mid-tropospheric flow<sup>7</sup>, implied a propagation velocity of 6-7 m s<sup>-1</sup> towards 280°, that is with a significant component parallel to the front. This is consistent with the sequence of cloud photographs and suggests that the feature would be carried over the southern part of the triangle at about 23.00 GMT. The weather logs of the JASIN ships revealed that thunderstorm activity had been reported by three ships in that region between 21.00 GMT and midnight (see Fig. 4 for a synoptic chart).

We conclude that the anomalous SASS winds occurred in a region where deep mid-level convection was taking place. Possible explanations for the unrealistic satellite winds are: (1) that the regions of high backscatter (Fig. 2) are associated with increasing ocean surface roughness due to locally high winds in the vicinity of the convective cells which are small compared with the SASS resolution cell  $(18 \times 70 \text{ km})$ ; (2) the large raindrops in the storm may significantly modify the ocean surface

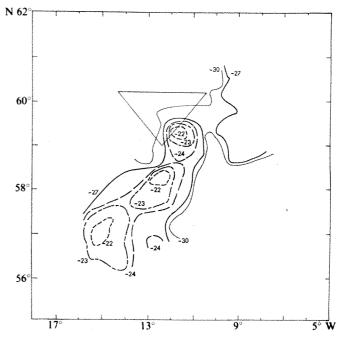


Fig. 2 Contours of normalized backscatter intensity  $(\sigma^\circ)$  obtained from part of the left-hand backward viewing SASS beam in the vertical polarization mode. Rev 557, 23.16 GMT 4 August 1978. Units are Db. Values of  $\sigma^\circ$  have been corrected for variation with incidence angle. To deduce wind direction data from two beams are combined with a consequent loss of areal coverage, hence the difference between Figs 1 and 2.

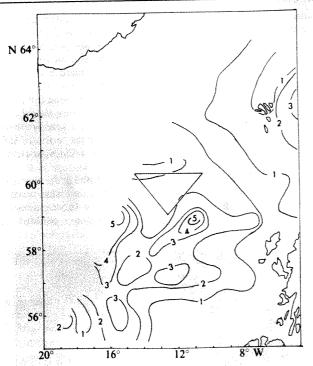


Fig. 3 Contours of vertically-integrated liquid water (units in 0.1 kg m<sup>-2</sup>) for Rev 556, 21.33 GMT 4 August as deduced from SMMR.

roughness, thereby invalidating the relationship between  $\sigma^{\circ}$  and wind vector; and (3) the rain may be responsible for additional backscatter in the atmosphere.

Concerning explanation (1), although thunderstorms<sup>8,9</sup> can produce large gusts at the surface through the evaporation of precipitation into the downdraught, on this occasion there was no evidence from surface data sampled at 1-min intervals that gusts exceeded 8 m s<sup>-1</sup> which is consistent with the observed thermodynamic structure.

For explanation (2), recent radar scattering experiments (Moore, personal communication) conducted in a wind/wave

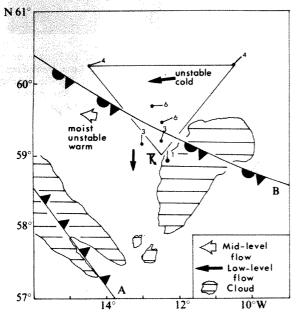


Fig. 4 Synoptic chart showing surface winds in the JASIN area (speeds in m s $^{-1}$ ) and the positions of fronts A and B at 22.00 GMT. Areas of deeper cloud, as identified from Seasat VIRR imagery, are also indicated together with information on the characteristics of the flow at mid-levels obtained from radiosonde data in the JASIN area.

tank have shown that the backscatter from small-scale surface roughness produced by artificial rain predominates for light wind conditions (<10 m s<sup>-1</sup>). To account for the large  $\sigma^{\circ}$ , the affected area would have to be several tens of km2 with moderate rain rates (5-10 mm h<sup>-1</sup>).

Finally, for explanation (3), calculations show that the large  $\sigma^{\circ}$ could be the result of backscatter from a single, localized rain cell. Assuming a thunderstorm cell size of 80 km<sup>2</sup> and a vertical rain column of 5 km would require rain rates of ~12 mm h account for the observed  $\sigma^{\circ}$ . Surface observations from ships near the anomaly indicate mainly light rain (0-2 mm h<sup>-1</sup>) but with heavy rain showers at one ship from 23.32-23.45 GMT. It seems that the anomaly was caused by either direct or indirect effects of rain or a combination of the two. Other factors which might lead to high backscatter are hail, which, because of its extremely limited extent is unlikely to have been observed from the ship array, and h.f. interference due to lightning

Further work is necessary to clarify why SASS fails to give realistic winds. This is of special interest if a combination of different Seasat sensors and ground-based observations indicate that the anomaly is related to an actual atmospheric phenomenon (as above) which otherwise might not be detected. Detailed analysis of the combined JASIN/Seasat data set should improve our understanding of air-sea interaction and the links between the near-surface layers and larger scales.

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# **Determinations by Seasat of** atmospheric water and synoptic fronts

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The Seasat Scanning Multichannel Microwave Radiometer  $(SMMR)^{1,2}$  determined the total atmospheric water vapour,  $q_0$ over 600-km wide swaths with a resolution of 54 km. Using radiosonde data from the Joint Air-Sea Interaction experiment, JASIN<sup>3</sup>, we show here that the SMMR  $q_t$  distributions can be used to detect the position of atmospheric fronts in the lower troposphere. Unlike visible and IR radiometry these SMMR determinations are not hampered by extensive cirrus or by lack of frontal cloud. Advantages of the SMMR over previous passive microwave instruments on satellites such as Nimbus 5 and 6 (refs 4-9) are: the use of more channels, allowing better discrimination between the effects of liquid water, water vapour and sea state; and improved spatial resolution. The SMMR performance has been evaluated at several workshops 10-14. Our results 15 show that the SMMR  $q_i$  determinations have similar accuracy to in situ radiosonde measurements.

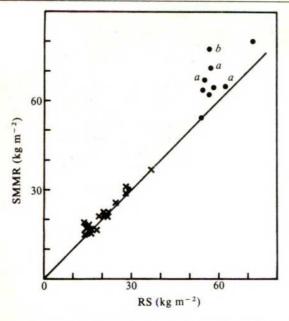


Fig. 1 Comparison of integrated atmospheric water vapour  $q_t$  as measured by the SMMR and by radiosondes.  $\times$ , JASIN values;  $\odot$ , tropical soundings. a indicates tropical soundings for which SMMR values may be too high due to the island of Guam being within the field of view. b, SMMR values may have been affected by heavy rain.

Independently evaluated SMMR and radiosonde estimates of  $q_t$  were compared at the Seasat-JASIN Workshop<sup>13</sup> (Fig. 1). The SMMR results were evaluated using the Wentz algorithm<sup>10</sup>, the nearest grid values being linearly interpolated to the radiosonde launch position. Radiosonde data from the JASIN experiment, conducted in the North Atlantic (60 °N, 12 °W) during July to September 1978³, are shown. Specially calibrated fast sampling radiosondes were used and comparisons restricted to flights launched within  $\pm 2$  h of Seasat overpass time. Estimated accuracy of the radiosonde  $q_t$  value was  $-\pm 1.7$  kg m<sup>-2</sup> (ref. 13). The mean difference, SMMR minus radiosonde, was  $1.2\pm 1.6$  kg m<sup>-2</sup>. To provide comparison at higher  $q_t$  values

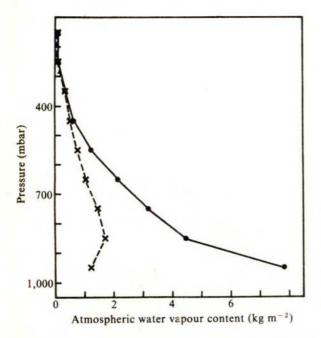


Fig. 2 Mean atmospheric water vapour content of 100-mbar layers during JASIN. Solid line shows the mean value and the dashed line the standard deviation of individual observations for some 200 radiosonde flights of which 110 reached 400 mbar.

radiosonde flights from tropical islands and weather ship Tango are also plotted. These are standard synoptic ascents within 3 h of overpass time. Estimates of  $q_t$  cannot be obtained over land and the tropical island of Guam, being significant in size compared with the SMMR resolution, may have affected the data for three flights marked. One comparison may be affected by local heavy rain. Eliminating these flights the mean difference is  $4.9 \pm 3.7$  kg m $^{-2}$ . Radiosonde errors would be expected to be greater in these ascents and, as for JASIN, the actual error due to the SMMR is not known. However, changes to the SMMR evaluation procedures following the Seasat–JASIN workshop have shown reduced biases<sup>14</sup>.

The SMMR also provided liquid water and rain rate estimates; however, no quantitative evaluation was possible because of the difficulty of obtaining reliable *in situ* measurements. The horizontal variation of liquid water content was

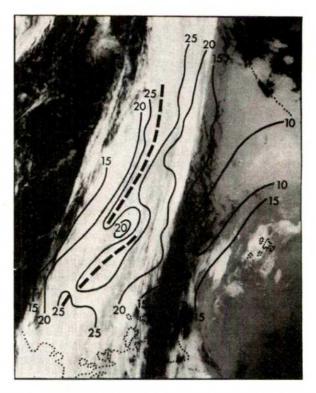


Fig. 3 NOAA 5 IR satellite photograph at  $10.37\,\mathrm{GMT}$  on 5 September 1978 showing a front stretching from Ireland and Scotland (lower left) to Iceland (upper right). SMMR derived values of  $q_{\rm t}$  are shown in kg m<sup>-2</sup>. The dashed line marks the maximum in these values. (Photograph: University of Dundee.)

qualitatively consistent with the meteorological conditions (see ref. 16). For JASIN, SMMR indicated rain when precipitation was 'widespread'—reported by several JASIN ships. Light scattered showers were not detected probably because such showers have horizontal dimensions very much less than the SMMR resolution. Although difficult to obtain  $^{17}$ , quantitative determination of rainfall would have obvious meteorological value. Rain detection is also needed to flag doubtful  $q_t$  values (Fig. 1). However, two different rain rate algorithms used in SMMR evaluation gave different quantitative results showing that much more work is needed on this variable.

The accurate horizontal distributions of  $q_t$  provided by SMMR are useful not only for the calculation of atmospheric water budgets<sup>18</sup>, but also for determining the position of synoptic scale fronts in the lower troposphere. To demonstrate this we need to consider the vertical distribution of water vapour in the atmosphere. Figure 2 shows the mean water vapour content of each 100-mbar atmospheric layer for the whole of the JASIN experiment. The water vapour content falls rapidly with

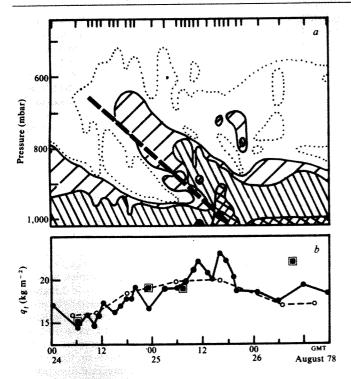


Fig. 4 a, Time-height atmospheric cross-section showing the variation of specific humidity with the passage of a warm front. Values shown are 2 g per kg (dotted), 4-6 g per kg (wide diagonals), 6-8 g per kg (close diagonals), above 8 g per kg (crosshatched). The section is based on 31 radiosonde flights at the times marked at the top. The dashed line marks the approximate region of the frontal surface. b, Values of  $q_t$  from the radiosonde data (solid line), SMMR (■) and SMMR q<sub>t</sub> distribution at 07.30 GMT on 24 August. The moist air ascending the front results in an increase in  $q_t$  with time to a maximum at about the time of surface frontal passage. Following the front there is a decrease to the warm air boundary layer value.

decreasing pressure and temperature, ~80% of the total occurring in the lowest 250 mbar. In contrast the variability of water vapour content is a maximum not at the surface but in the 900-800 mbar layer, and decreases less rapidly, 80% of the variation being spread over the lowest 400 mbar. Thus for this mid-latitude area, although the surface boundary layer is typically 1-2 km deep and contains much of the water vapour, the variability of  $q_t$  is not dominated by boundary layer processes but reflects changes in humidity at levels up to 5 km. Synoptic scale fronts cause moistening at these levels and hence result in significant variations of  $q_t$ . For example, Fig. 3 shows a NOAA 5 satellite IR image in which an occluded front is marked by a 250-km wide band of cirrus which masks any lower level clouds. The variation of  $q_t$  measured by SMMR showed a strong maximum associated with the front, marked in Fig. 3 by the broken line. The position of this maximum was well defined to within the 54-km SMMR resolution, except in one area where a more complicated frontal structure was indicated.

The actual position of the surface front relative to the  $q_t$ maximum will vary from case to case. As an example Fig. 4a shows a radiosonde derived time-height cross-section of specific humidity during the passage of a warm front. Although the front was a significant feature of the radiosonde data, lack of frontal cloud prevented its detection in visible or IR satellite images. The corresponding variation of  $q_t$  is shown in Fig. 4b. SMMR values are in reasonable agreement with the radiosonde values. A difference on the 26 August was probably caused by larger values of  $q_t$  occurring just outside the JASIN area but within the SMMR resolution cell. Radiosonde observations from other JASIN ships show that the front propagated through the area with little change in structure and hence the SMMR horizontal q distribution for 07.30 GMT on 24 August has been transformed

to the time variation shown by the dashed line in Fig. 4. Both radiosondes and SMMR show that, in spite of a marked reduction in boundary layer height, there was a gradual increase of  $q_i$ with the approach of the front. The surface passage of the front was marked by a sharp decrease of  $q_i$  to the warm air boundary layer value. By mapping these  $q_t$  variations in the SMMR data from several Seasat orbits the changing position and alignment of the front have been determined more precisely than was possible using the conventional meteorological observations.

Detection of synoptic fronts in the lower troposphere using SMMR  $q_1$  measurements offers an important new aid for meteorological analysis which is already proving valuable in the interpretation of the JASIN data.

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# **Anomalous magnetic directions** recorded by laboratory-induced chemical remanent magnetization

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It has been generally accepted that chemical remanent magnetization (CRM) is acquired either in the direction of the ambient field at the time of chemical alteration or in the direction of a pre-existing natural remanent magnetization (NRM)<sup>2-4</sup>. We report here our experiments in which CRM is carried by magnetite formed during laboratory heating of titanomaghaemite in submarine basalts. This CRM is a stable, single component remanence, acquired in an intermediate direction between the ambient field and pre-existing NRM direction. The role of prior NRM in controlling the direction of CRM is suppressed by externally applied fields of  $\approx 50 \mu T$ . (0.5 Oe) but is prominent in fields of  $\approx 20 \,\mu\text{T}$ . Thus, stable, intermediate-direction CRMs acquired in past geomagnetic fields are a possibility.

CRM was induced in samples from Site 332B of Leg 37 of the Deep Sea Drilling Project. The chemical alteration that occurs when these basalts are heated above 200 °C has been well documented<sup>4-7</sup>. principal mineral. The magnetic titanomaghaemite, breaks down forming an intergrowth of a strongly magnetic titanium-poor titanomagnetite, near magnetite in composition and a titanium-rich phase with a composition close to ilmenite. The NRMs of our samples were moderately strong ( $> 0.1 \text{ A m}^{-1}$ ). The Curie temperatures lay between 330 and 365 °C before the laboratory heating. After the samples were heated their Curie temperatures were close to

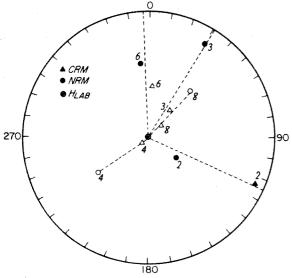


Fig. 1 Equal area plot showing the direction of CRM relative to the laboratory field and the initial NRM direction. Solid (open) symbols denote positive (negative) inclinations. CRM was induced in a 20  $\mu T$  (0.2 Oe) laboratory field in samples 2 and 4 and in a 10  $\mu T$  (0.1 Oe) field in samples 3, 6 and 8. The CRM direction is displaced away from the laboratory field direction towards the NRM direction.

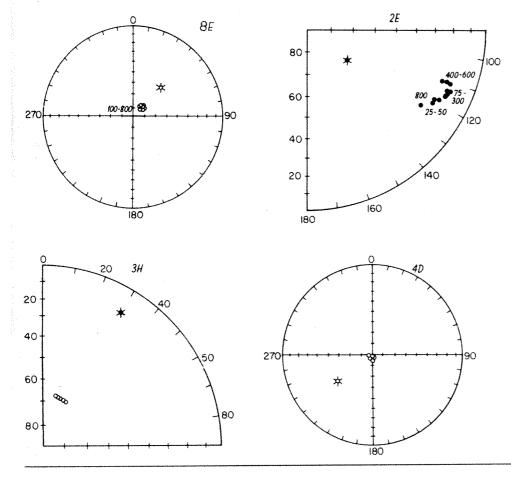
 $580\,^{\circ}\mathrm{C}$ , indistinguishable from the value for pure magnetite. Hysteresis parameters measured before heating indicate that the titanomaghaemite is present as large ( $\approx 20~\mu\mathrm{m}$ ) pseudosingle-domain grains. (The ratio of saturation remanence to saturation magnetization,  $J_{\rm rs}/J_{\rm s}$ , ranges from 0.08 to 0.15.) The magnetite-like phase, which is produced during heating, is present as very fine single-domain grains, having  $J_{\rm rs}/J_{\rm s}$  ratios between 0.33 and 0.36.

CRMs were induced in eight samples. Each 1 cm (o.d) by

1.5 cm long cylindrical sample was divided into two sub-samples before CRM was induced. The initial NRM was left intact in the first specimen while the second was demagnetized by tumbling in a peak alternating field of 100 mT. The use of two sub-samples allowed us to study separately the roles of the external field and the NRM in determining the direction of CRM. CRM was produced by simultaneously heating both specimens slowly ( $\approx 100~^{\circ}\text{C h}^{-1})$  in vacuo (10 $^{-2}$  torr) to a temperature of 515  $^{\circ}\text{C}$  which was then held for 24 h. Fresh samples were used during successive heatings in laboratory fields of 0, 10, 20 and 50  $\mu\text{T}$ . The fields were applied along the cylindrical axis of each specimen without regard to the NRM direction of the sample. After heating, the samples were cooled in a null field ( $<10^{-2}~\mu\text{T}$ ) to avoid the acquisition of thermoremanent magnetization or TRM.

CRMs acquired in 50 µT all lay along the direction of the laboratory field. The presence of an initial NRM had no apparent effect on the direction of CRM. Strikingly different results were obtained with smaller inducing fields (Fig. 1). In five of the eight samples studied, the CRM in samples with an initially intact NRM did not lie in the laboratory field direction. The CRM directions in these samples were deflected by as much as 110° from the laboratory field direction towards the initial NRM direction. The CRM in samples which were initially demagnetized always lay in the external field direction. All the CRM directions were stable in alternating fields up to 100 mT (Fig. 2). The CRM produced with an intermediate direction is therefore a single, directionally stable component and is not a vector sum of components in the laboratory field and NRM directions. Slight initial directional changes on demagnetization are attributed to the removal of viscous components acquired by fine grained magnetite near superparamagnetic size. The CRMs were magneticaly hard with median demagnetizing fields (MDFs) of 57-68 mT (Fig. 3). This contrasts with the magnetically soft NRM of the parent titanomagnaemite in which MDFs ranged from 11 to 15 mT.

Alternating field demagnetization curves of CRM were compared with those of saturation isothermal remanence



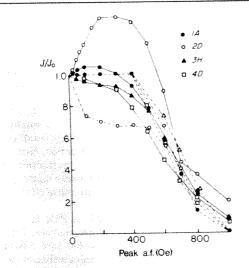


Fig. 3 Intensity of CRM and ARM during a.f. demagnetization. CRM is given by a solid line, ARM by a dashed line.

(SIRM)-and also of anhysteretic remanent magnetization (ARM) acquired in biasing fields of 50 µT and 100 mT a.f. Both the ARM and the SIRM were partially thermally demagnetized by a rapid heating to 515 °C in a null field before a.f. demagnetization was begun. This allowed us to compare the coercivity spectra in the same blocking temperature range for each type of remanence. The a.f. demagnetization curves of CRM, ARM and SIRM are essentially the same at a.f. levels above 40 mT. It would, therefore, be difficult to identify the CRM on the basis of a comparison of coercivity spectra. Differences in the portion of the coercivity spectra below 40 mT result from VRM acquired between the acquisition of CRM and its subsequent measurement. The ARM was demagnetized immediately after acquisition and the samples had little time in which to acquire VRM.

As further chemical alteration accompanied heating to temperatures above 515 °C, a direct comparison of the properties of TRM and CRM in the 515-580 °C blocking temperature range was not possible.

We conclude that both the ambient magnetic field and preexisting NRM have a role in determining the direction of CRM. In ambient fields of <50 µT the CRM may lie in a direction between the NRM direction and the ambient field direction. Neither of these directions can be recovered by techniques such as vector subtraction of a.f. demagnetization data.

CRM of this type will be difficult if not impossible to distinguish from other remanences on the basis of a.f. demagnetization curves. A direct comparison of CRM with TRM coercivity spectra was not possible because further chemical alteration accompanies heating above 515 °C, but we have found that the a.f. demagnetization characteristics of the CRM are very similar to those of ARM which is often considered an analogue of TRM<sup>8</sup>

It cannot therefore be assumed that CRM will faithfully record the geomagnetic field direction either at the time of the sample formation or when they alter later. A particularly misleading result could arise when chemical alteration occurs after a geomagnetic polarity reversal. The presence of both NRM and a reversed geomagnetic field could result in a single chemical magnetization component nearly perpendicular to the geomagnetic field direction. This mechanism could be a contributing factor in the anomalously shallow palaeomagnetic inclinations often observed in deep drilled marine basalt samples9.

How general are these conclusions? If the direction in which CRM is acquired is partly controlled by magnetostatic interaction with a pre-existing NRM, then the proximity of the parent and daughter magnetic carriers and the intensity of the NRM relative to the external field are critical. If the coupling between the NRM and the CRM is magnetostatic, the moderately high

temperatures used in our experiment will have reduced the influence of the NRM because of the temperature dependence of the spontaneous magnetization of the parent magnetic minerals. In both the low temperature oxidation undergone by submarine basalts and the high temperature alteration of titanomaghaemite which is described here, and which can occur naturally in subaerial basalts10, the parent and daughter magnetic minerals are closely situated and both are strongly magnetic. Thus, in these cases anomalous CRM directions may be common. If CRM is produced in a system where the parent magnetic mineral is essentially non-magnetic such as when magnetite is grown during the serpentinization of olivine, the NRM of the sample as a whole may be of little importance in controlling the CRM direction.

Our present results do not enable us to decide for or against the magnetostatic model of CRM directional control. Current experiments on CRM acquisition during the reduction of weakly magnetic haematite to magnetite may help us resolve this ques-

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### Isotopic and biostratigraphical records of calcareous nannofossils in a Pleistocene core

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Knowledge of the species composition is essential for the isotopic record of polyspecific coccolith assemblages in deep-sea sediments to be interpreted properly. We have determined the carbon and oxygen isotopic composition as well as the relative abundance of coccolith species on the 3-25 µm fractions of the Caribbean core P6304-4. The dominant taxon in all samples is well-preserved Gephyrocapsa spp. Oxygen isotope variations clearly define glacial-interglacial oscillations. Except in the upper part of the core, carbon isotopic compositions are not related to the oxygen isotope stratigraphy. The occurrence of three coccolith datum levels in the core correlates almost exactly with globally-synchronous horizons1. The amplitude of the coccolith  $\delta^{16}$ O record (2.4%) is significantly greater than that for the planktonic foraminifera Globigerinoides sacculifer in P6304-4 and adjacent cores<sup>2-4</sup>. We suggest here that selective dissolution or deep calcification during interglacials reduced the amplitudes of G. sacculifer in these cores and that the coccolith record is a more reliable indicator of temperature and  $\delta^{18}O$ changes in surface seawater.

Calcareous nannofossils (dominantly coccoliths) comprise about half of the CaCO<sub>3</sub> in deep-sea sediments<sup>5</sup>. Because of their abundance, shallow and restricted depth habitat<sup>6</sup>, wide geographical distribution<sup>6</sup>, and dissolution-resistant characteristics<sup>7-9</sup>, the stable isotope record of calcareous nannofossils

should complement other techniques in palaeooceanographic investigations.

The oxygen and carbon isotopic composition in coccolith-rich fractions from sediments ranging in age from Upper Cretaceous to Recent have been used as indicators of sea-surface conditions 10-17. Results suggest that coccolith fractions yield isotopic records which are generally consistent with data on foraminifera for the same time interval (and often on the same core material). Nonetheless, a major problem remains in interpreting coccolith isotopic compositions: because of their very small size, coccoliths can only be concentrated from deep-sea sediments as polyspecific assemblages. Because species-dependent nonequilibrium isotopic fractionation in coccolith secretion has been demonstrated<sup>18,19</sup>, it is possible that variations in species abundance will obscure the palaeoenvironmental significance of the isotopic signal from coccolith assemblages. To evaluate this possibility and to test the usefulness of coccolith isotope stratigraphy in Pleistocene sediments, we have determined the isotopic composition and coccolith taxonomy in the fine-fraction of core P6304-4 from the Caribbean.

Core P6304-4 is one of four long piston cores from the same area of the central Caribbean ( $\sim$ 15° N,  $\sim$ 70° W, 3,930-4,140 m in depth) on which Emiliani<sup>2,3</sup> has reported the oxygen isotope record of G. sacculifer. We separated the 3-25  $\mu$ m fraction of bulk sediment samples taken at 10-cm intervals by settling and short centrifugation techniques<sup>20</sup>. High-resolution light microscopy and scanning electron microscopy were used to estimate

the relative abundance and state of preservation of 34 coccolith species. Details of our taxonomic data will be given elsewhere (T.F.A. and J.C.S., in preparation). These results indicate that the carbonate fraction in the 3–25 µm fractions consists almost exclusively of well-preserved coccoliths. The most abundant taxon at all depths was the genus Gephyrocapsa; G. caribbeanica and G. oceanica were dominant (Fig. 1). Emiliania huxleyi, an abundant species during the past 270,000 yr (refs 1, 21), was absent or rare in our 3–25 µm fractions (but was abundant in the bulk sediment samples). Because of its relatively small size, E. huxleyi was not retained in the 3–25 µm fraction. Total (coccolith) carbonate makes up only 10–50% of this fraction. X-ray diffraction analysis indicates that the non-carbonate portion consists of quartz, chlorite and/or kaolinite, mica (illite?) with occasional feldspar and smectite.

The samples were treated with 100% H<sub>3</sub>PO<sub>4</sub> at 25 °C to evolve CO<sub>2</sub> for mass-spectrometric isotope analysis. Pretreatment consisted of soaking the bulk sediment in a 5% clorox solution before size fractionation. The samples were not vacuum roasted as initial experiments indicated that this did not improve the precision of replicate analyses ( $\pm 0.04$  for  $\delta^{13}$ C),  $\pm 0.06$  for  $\delta^{18}$ O) nor alter significantly the isotopic composition of these coccolith fractions. The mass-spectrometric data are reported in  $\delta$ -terminology as % deviations from the PDB standard<sup>22</sup>.

The oxygen isotope results show the characteristic 'saw-toothed' pattern of glacial-interglacial oscillations down to at least isotope stage 14 (Fig. 1). The sharp glacial-to-interglacial

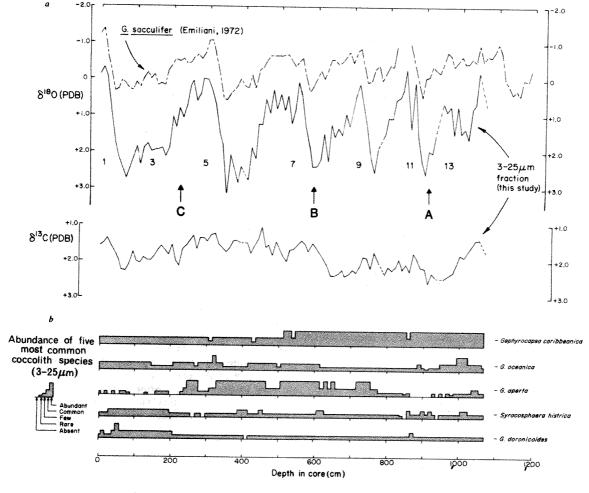


Fig. 1 The oxygen and carbon isotopic results on the 3-25 μm fraction of core P6304-4. Emiliani's² oxygen isotope data on G. sacculifer is also shown. The numbers beneath the 3-25 μm δ¹8O curve identify the interglacial isotopic stages³. In a, A, B and C indicate the globally-synchronous late Quaternary coccolith datum levels of Thierstein et al.¹. A, extinction of Psuedoemiliania lacunosa (458,000 yr BP) at 910 cm; B, first appearance of E. huxleyi (263,000 yr BP) at 590 cm; C, reversal in dominance of G. oceanica and E. huxleyi (85,000 yr BP in tropical and subtropical cores) at 220 cm. The relative frequency of the five most abundant coccolith species in the 3-25 μm fraction (b) was determined from the examination of about 100 fields of view on smear slides⁴5. Abundant, 10-100 specimens per field of view; common, 1-10 specimens per field of view; few, single specimen in 1-10 fields of view; rare, single specimen in 10-100 fields of view.

**Table 1**  $\delta^{18}$ O maxima and minima (stages 1-12) for G. sacculifer from Caribbean cores<sup>2-4,35</sup>

	zubic .				•		
Isotope stage	A172-6 4,160 m 14°59' N 68°51' W	P6304-4 4,136 m 15°27' N 70°43' W	P6304-9 4,126 m 14°57' N 68°55' W	P6304-7 3,929 m 15°06' N 69°48' W	P6304-8 3,927 m 14°59' N 69°20' W	A179-4 2,965 m 16°36' N 74°48' W	V12-122 2,800 m 17°00' N 74°24' W
1	-1.87	-1.30	-1.36	-1.38	-1.29	-1.79	-2.18
ż	-0.05	+0.30	+0.43	+0.29	+0.59	+0.12	0.00
5	-1.39	-1.05	-1.36	-1.32	-1.34	-1.65	-1.69
6	-0.10	+0.57	+0.39	+0.30	+0.43	+0.11	+0.24
7	-1.41	-0.67	-1.22	-1.15	-1.15	-1.63	-1.53
8	-0.23	+0.25	+0.39	+0.11	+0.34	+0.25	-0.03
9	-1.42	-0.81	-1.23	-1.49	-0.89	-1.60	-1.86
10	+0.03	+0.07	+0.22	+0.21	+0.32	-0.13	-0.06
11	-1.08	-0.95	-0.82	-1.14	-0.72	-1.40	-2.14
12	(-0.62)	+0.49	+0.21	+0.41	+0.47	(-0.71)	+0.33
$\delta$ igl	-1.4	-1.0	-1.2	-1.3	-1.1	-1.6	-1.9
gigi Sal	-0.1	+0.3	+0.3	+0.2	+0.4	0.0	0.0
$ar{\delta}$ gl $\Delta \delta$	1.3	1.2	1.5	1.5	1.5	1.6	1.9

 $\bar{\delta}$ igl, Mean interglacial.  $\bar{\delta}$ gl, Mean glacial.  $\Delta\delta$ , Average glacial-interglacial amplitude for adjacent stages.

transitions are well-defined. These stage boundaries in the coccolith fraction occur 10 ( $\pm 15$ ) cm lower than in Emiliani's G. sacculifer record<sup>2</sup>. Given that this difference is about the same as our sampling resolution, the agreement is excellent. In addition, the occurrence of three coccolith biostratigraphic markers relative to the coccolith  $\delta^{18}$ O curve is in almost exact agreement with their correlation in nine other deep-sea sediment cores to the late Quaternary oxygen isotope chronostratigraphic framework<sup>1,23</sup>. The agreement is slightly better for the coccolith record than for the foraminifera record. This correlation between biostratigraphic and isotopic results is convincing evidence for the reliability and precision of the stratigraphical information in the  $\delta^{18}$ O record of coccoliths.

Variations in the carbon isotopic composition of the coccolith fraction (+1.1 to 2.6%) are not obviously related to the oxygen isotope signal. A possible exception is in the interval of the most recent deglaciation, where  $\delta^{13}$ C values decrease by 0.8% from the Upper Pleistocene to the Holocene. A similar carbon isotope shift is recorded in planktonic foraminifera from the Caribbean core V12-122<sup>24</sup>. These results suggest upwelling of nutrient- and  $^{12}$ C-rich waters was higher in the Caribbean during the Holocene than during the last glacial maximum; this is opposite to changes in vertical circulation inferred from carbon isotope evidence in cores from the eastern subtropical Atlantic<sup>25</sup> and the western equatorial Pacific<sup>14</sup>.

Notwithstanding the obvious similarity in isotopic stratigraphy, there are two prominent differences between the  $\delta^{18}$ O signals of the coccolith fraction and G. sacculifer in core P6304-4. First, coccoliths are enriched in <sup>18</sup>O relative to G. sacculifer by 1 to over 2%. Part of this discrepancy could be due to differences in standard calibrations and sample preparation techniques between laboratories. On the other hand, this difference is consistent with data on oxygen isotopic fractionation in these taxa. G. oceanica grown in culture experiments is enriched in <sup>18</sup>O with respect to equilibrium <sup>19</sup>. The question of whether G. sacculifer deposits its test at oxygen isotope equilibrium with near-surface seawater has not been resolved. G. sacculifer assemblages from well-preserved Holocene sediments are often close to isotopic equilibrium 2-4,14,26,27 In contrast, living G. sacculifer populations collected in plankton tows are depleted in <sup>18</sup>O relative to isotopic equilibrium with ambient seawater<sup>24,28-31</sup>. In either case, provided that the isotopic properties of G. oceanica are representative of the coccolith fraction in P6304-4, then the direction of the difference in  $\delta^{18}$ O between this fraction and G. sacculifer is reconciled. (Comparable differences between coccoliths and planktonic foraminifera have been reported previously 13-16.)

Second, the amplitude of glacial-interglacial fluctuations averages 2.4% for the coccolith fraction through stage 12 but only 1.2% for G. sacculifer. Inasmuch as this shallow-dwelling foraminifera occupies the same approximate depth habitat as coccolithophores<sup>26,27,32</sup>, both carbonate constituents should

have been exposed to the same temperature and oxygen isotope composition of seawater during climatic cycles and therefore should have recorded the same  $\delta^{18}$ O fluctuations. Obviously the isotopic record of one (or perhaps both) of these carbonates has been affected systematically by one or more other factors such as variations in non-equilibrium isotope fractionation, changes in depth habitat, and isotopic bias due to selective dissolution<sup>33</sup>.

It is possible that changes in the abundance of the most common species contributed to the glacial-interglacial  $\delta^{18}$ O fluctuations of the coccolith fraction. Our taxonomic study proves that they did not (Fig. 1). Gephyrocapsa is the dominant taxon in the 3-25 µm fraction throughout the core. There is no obvious correlation between the abundance of the most common species of this genus, G. caribbeanica, G. oceanica, and G. aperta (and the next most abundant taxa) and the  $\delta^{18}$ O signal of this fraction. Variation in depth habitat of coccolithophores between glacial and interglacial times is implausible. For this effect to operate, coccolithophores would have had to migrate to deeper waters during glacials. However, as they are photosynthesizers their depth habitat is restricted to the photic zone regardless of climate-induced changes in the physical properties of surface seawater. For the same reason, selective dissolution of coccoliths (for which there is no evidence in our samples) would not result in a bias towards an <sup>18</sup>O-enriched sediment assemblage as it often does in shallow-dwelling planktonic foraminifera<sup>27,32-34</sup>. The effect of differences in seasonal temperature contrast between glacial and interglacial times on the  $\delta^{18}$ O record of coccoliths is difficult to evaluate but was probably not of major importance. Temperature estimates based on quantitative faunal analyses 35-37 suggest that seasonal contrasts during glacials (4-5 °C) was greater than during interglacials (1-2 °C) due to greater variations in winter temperatures. The corresponding maximum seasonality effect on the  $\delta^{18}{\rm O}$  range of coccoliths (secretion during glacial winters and interglacial summers) would be  $\sim +0.4\%$ . The actual seasonality influence was probably much smaller. These considerations suggest that the oxygen isotope variation in the coccolith fraction from P6304-4 records primarily climate-induced fluctuations in the temperature and isotopic composition of Caribbean surface seawater.

If this is correct, then the amplitude of the  $\delta^{18}$ O signal in G. sacculifer from P6304-4 has been truncated. The isotopic record of G. sacculifer in other Caribbean cores analysed by the University of Miami<sup>2-4</sup> are generally consistent with the results on P6304-4 (Table 1, columns 1-6). However, the amplitudes of the  $\delta^{18}$ O signal in the five deeper cores (>3,900 m), including P6304-4, are smaller than the amplitudes recorded in two shallower cores (<3,000 m). This difference in amplitude is due mostly to less negative interglacial  $\delta^{18}$ O values in the deeper cores versus the shallower cores. The amplitude of the coccolith isotope signal in P6304-4 (2.4%) is more compatible with the results from V12-122. In fact, the  $\delta^{18}$ O signal predicted from

the isotopic and faunal temperature data on this core<sup>35</sup> together with experimental fractionation results on Gephyrocapsa very good agreement with our analyses (unpublished data).

The differences in the oxygen isotope record of G. sacculifer among these Caribbean cores, first noted by Shackleton et al. is not easily explained. The relationship between  $\delta^{18}$ O amplitude and depth would suggest that selective dissolution of G. sacculifer during interglacial epochs resulted in a variable proportion of <sup>18</sup>O-enriched tests in the carbonate fraction from which the samples were picked. Shackleton and Opdyke<sup>23,38</sup> report a similar relation between the  $\delta^{18}$ O record of G. sacculifer and depth in two cores from the western equatorial Pacific. However, differential dissolution causing a significant increase in the  $\delta^{18}$ O of G. sacculifer should also have been obvious in the state of preservation of the coccoliths (J. S. Killingley, personal communication). In addition, poor preservation of foramineral assemblages and low carbonate contents in Quaternary Caribbean and equatorial Atlantic cores are more pronounced during glacial than interglacial stages<sup>39-42</sup>. An alternative explanation may involve glacial-interglacial differences in the range of depth habitats and hence the mean temperature of test secretion 31,42-44. For example, Duplessy et al. 31 have reported recently that late-stage calcification of G. sacculifer during descent into cold waters significantly enriches fossil tests in <sup>18</sup>O relative to their living counterparts and note that this effect can produce sedimentary assemblages coincidentaly close to isotopic equilibrium with near-surface seawater. We emphasize that oxygen isotopic variations in Pleistocene coccoliths not only provide an excellent stratigraphic record but also may be a more reliable indicator of the temperature and  $\delta^{18}$ O of surface seawater than planktonic foraminifera. In fact coccolith  $\delta^{18}$ O variations may prove to be important for evaluating the extent and effects of the dissolution and/or deep-water calcification of planktonic foraminifera.

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# Extensive regulatory capabilities of a *Drosophila* imaginal disk blastema

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Every organism develops body patterns that are accurately repeated among individuals of the same species. To understand how cells interact to form these patterns, many investigators have studied the re-establishment of patterns following genetic and/or physical perturbations. In regulating systems, missing structures may be replaced by cell proliferation (epimorphosis) or by respecification without growth (morphallaxis)1. Epimorphosis involves the formation and growth of a blastema<sup>2</sup>, defined for vertebrate regeneration as a zone of proliferating cells, localized near the wound surface3, which becomes capable of regenerating even when isolated from the rest of the limb4. Clonal analysis of Drosophila imaginal disk fragments indicates that during pattern regulation most dividing cells are located near the wound surface<sup>5</sup>. Furthermore, mitotic figures (L. C. Abbott, personal communication) and cells incorporating <sup>3</sup>Hthymidine<sup>6</sup> are clustered near the wound surface of regulating fragments. Here we describe experiments involving cultures of this zone of proliferating cells, and report that it can regulate in isolation. These observations provide evidence that regulation of disk fragments is accomplished by growth of a blastema. Surprisingly, the isolated blastema regenerated more pattern elements than predicted by the polar coordinate model7. We resolve this paradox by proposing that regulation is initiated at free cut edges, where a blastema is formed that adds new positional values in a sequence. On completion of wound healing, blastema growth continues until remaining positional disparities are eliminated by intercalation, as presented in the polar coordinate model.

We used the property that imaginal disk tissue differentiates when transplanted into a metamorphosing larva to determine the fate of particular first leg disk regions (Fig. 1a, b). The three-quarter lateral plus endknob fragment (3/4L+EK)differentiated sectors A, B, C and D plus the tarsus. This region of the disk was subdivided into the 1/8LM and 5/8L+EK fragments, which produced sector D and sectors A, B and C plus the tarsus, respectively. Figure 1c gives the sectorized fate map of the disk obtained by pooling results from different fragments. The growth period of disk tissue is extended by transplantation into the abdomen of an adult female (culture in vivo). When 3/4L+EK fragments were cultured in adults and then differentiated in larval hosts, they usually duplicated the structures found in the fate map of the fragment (Fig. 1d). The 1/8LM fragment did not regenerate after 6 days in adult culture. An unusually high number of implants were not found (38/62), and the 11 differentiated implants only contained structures seen in the fate map of the fragment.

When the 3/4L fragment duplicates, the prospective sector D contains a zone of proliferating cells<sup>5</sup>. We isolated this area fragment I from 3/4L+EK fragments after 1 day of adult culture (Fig. 2a). The position of the nerve stalk and the

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Fig. 1 a, Structures differentiated by the first leg disk. To visualize all the adult structures the leg is displayed as if split open longitudinally. Disks differentiated all the segments and structures found in an in situ prothoracic leg. Donors and hosts (La) were late third-instar larvae. We divided the leg cuticle into sectors A-F to facilitate discussion of the results (see ref. 14 for a complete description of the markers). Each sector contained a minimum of two sector-specific markers. Pt = prothorax, Co = coxa, Tr = trochanter, Fe = femur, Ti = tibia, Ta 1 = first tarsal segment, Ta 2-5 = tarsal segments 2-5.\* Denotes position of the nerve stalk. Solid arrowheads symbolize metamorphosis. b, Fate mapping of disk regions. L = lateral, LM = lower medial, EK = endknob. The height of each column indicates the percentage of the cases that differentiated the particular sector. c, Sectorized fate map of first leg disk. d, Adult culture of 3/4L+EK fragments. Fragments were injected into the abdomens of fertilized, recently eclosed females (Ad); after 4 days of culture in vivo implants were transplanted into larval hosts. Reversed letters indicate duplicate sectors. Forty per cent of the 3/4L+ EK fragments only duplicated structures expected from the fate map, 53% also regenerated sector E and 7% only regenerated sector E, without duplicating any structures.

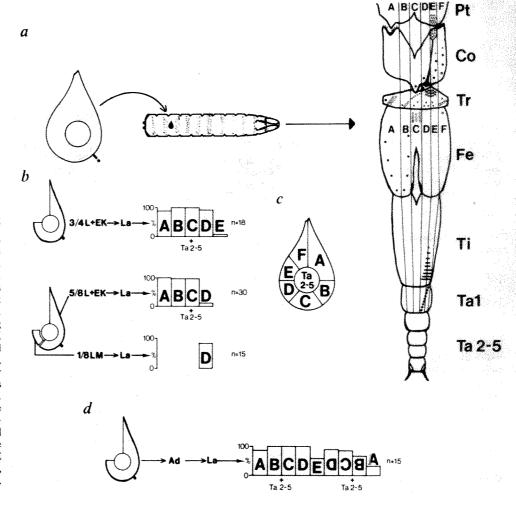
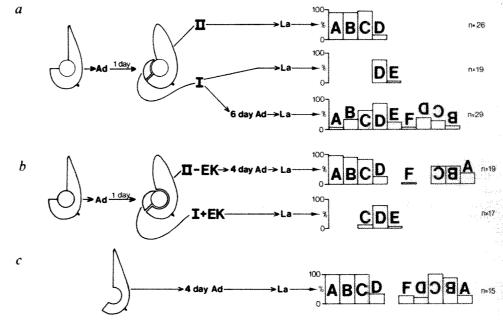


Fig. 2 a, Regulatory potential of the blastema region. 3/4L+EK fragments were cultured in vivo for 1 day; the implants were then cut into fragments I and II. Immediate metamorphosis was used to determine the fate of the II fragments. Only 15% of these cases differentiated sector D, whereas over 90% produced sectors A, B and C and Ta 2-5. I fragments were either injected into larval hosts or given an additional 6 days of adult culture before metamorphosis. Ta 2-5 appeared only after culture in vivo (in 21% of the cases). b, Regulatory potential of the II-EK fragment. 3/4L+EK fragments were cultured in vivo for 1 day, then I+EK fragments were injected into larval hosts while II-EK fragments were given another 4 days of adult culture before metamorphosis. Ta 2-5 were



differentiated by 94% of the I+EK fragments and 37% of the II-EK fragments. Forty-two per cent of the II-EK fragments only duplicated sectors A, B and/or C, 32% also regenerated sectors D and/or F, and 5% only regenerated sector D and/or F. II-EK fragments were used (instead of II fragments) to allow a direct comparison with the I fragment, which also did not include the endknob. Four days of culture were chosen to reduce the high frequency of transdetermination found in this fragment after 6 days of culture. c, Adult culture of 5/8L fragments. Fragments were given 4 days of adult culture before metamorphosis. Fifty-four per cent only duplicated sectors A, B and/or C, and 46% also regenerated sectors D and/or F. Ta 2-5 were differentiated by 60% of the cases. See Fig. 1 legend for a description of symbols.

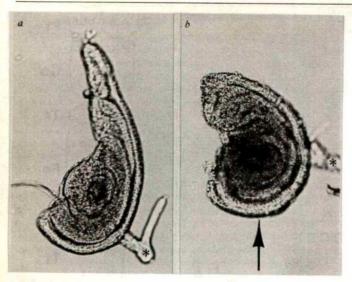


Fig. 3 3/4L+EK fragments immediately after fragmentation (a) and after 1 day in vivo culture (b). ×178. \* Denotes nerve stalk, arrow indicates position of second cut (see Fig. 2b). Note in b that the original cut surfaces have not fused.

presence of unfused cut surfaces facilitated orientation of the implants (Fig. 3). Immediate metamorphosis of fragment I yielded structures of sector D, but never sector C. The remaining piece (fragment II) predominantly differentiated sectors A, B and C plus the tarsus (Fig. 2a).

We determined the regulatory capabilities of regions within the 3/4L+EK fragment after 1 day of culture in vivo. I fragments were isolated, then given an additional 6 days in adult hosts (Fig. 2a). Although the amount of regulation varied, these fragments differentiated identifiable and organized patterns. Most (18/29 cases, 62%) contained structures that were never formed by uncultured I fragments (sectors A, B, C and/or F). Thirteen of these cases duplicated some or all of the structures present (for examples, see Fig. 4, cases 4 and 5). We also analysed these data in a pairwise manner to determine whether these surprising results were caused by cutting errors. Figure 4 shows cases in which both the I and II fragments from a single 3/4L+EK implant differentiated. In cases 1-3, fragment I produced sector D and showed some regeneration. In all the other paired cases I fragments extensively regenerated missing structures. They contained structures also found in the II fragment of the pair (boxed sectors), and in addition were able to differentiate structures that were not part of the 3/4L+EK fate map (sector F, cases 6-8). In case 8 the I fragment produced an entire leg! Thus, we conclude that the I fragments regenerated during culture in vivo.

II-EK and 5/8L fragments differentiated the same structures after transplantation into larvae (sectors A, B and C, data not presented) and therefore have the same fate. After 4 days in adults they regulated similarly; most cases duplicated and some of these fragments also regenerated (Fig. 2b, c). In contrast, the I and 1/8LM fragments had the same fate (sector D, Figs. 2a,1b), but only the I fragment regenerated after 6 days of adult culture. Therefore, during 1 day of culture in vivo of the 3/4L+EK fragment the regulative potential of only prospective sector D cells was altered. Furthermore, clonal analysis indicated that cell divisions increased significantly during regulation only in sector D of the 3/4L fragment<sup>5</sup>. We conclude that during 1 day of adult culture a blastema is formed in this region, and we propose that blastema formation and growth are integral steps in the regulation of disk fragments.

The extensive regulatory capabilities of the blastema region challenge current explanations of how imaginal disk fragments regulate. According to the polar coordinate model<sup>7</sup>, wound healing juxtaposes cells with disparate positional values; this stimulates growth, and intercalation proceeds until positional values are continuous. The 'rule of intercalation by the shortest

route' predicts the type of regulation a particular fragment will express. A fragment containing more than half the total number of positional values regenerates, whereas a fragment with less than half the values will only duplicate. The isolated blastema fragments violate two aspects of this rule. First, many I fragments were able both to regenerate and duplicate, which is inconsistent with the model<sup>8,9</sup>. Second, the I fragments consistently regenerated missing structures, and produced an entire leg in two of the cases given 6 days additional culture in vivo, although they contained few positional values. When the 3/4L + EK fragment was cultured in vivo for 1 day, on immediate metamorphosis no I fragment duplicated structures, and only 5% differentiated sector E in addition to D (Fig. 2a). According to the positional value map of the first leg disk<sup>10</sup>, sectors D and E contain much less than half the number of circumferential positional values.

A possible resolution of this problem is suggested by comparing the time course of wound healing with our data. An imaginal disk consists of a folded sheet of columnar epithelial cells and an overlying sheet of squamous epithelial cells (peripodial membrane), which form a continuous epithelium<sup>11</sup>. Reinhardt and Bryant<sup>12</sup> have shown that within 12 h of fragmenting a wing disk, the columnar epithelial cells at each cut surface make contact with cells of the peripodial membrane (heterotypic healing). By 1–2 days after fragmentation, columnar epithelial cells from the previously separated cut edges fuse (homotypic healing). The polar coordinate model<sup>7,12</sup> suggests that homotypic contacts initiate and control pattern regulation. However, we isolated blastemas with extensive regulative capacities before wound healing was complete, and therefore conclude that homotypic closure is not necessary for the initiation of regulation.

Two independent observations support this conclusion and further characterize the early blastema. First, mitotic figures are localized to the blastema region of the 3/4L+EK fragment within 1 day of adult culture (L. C. Abbott, personal communication). Thus, proliferation is stimulated before wound healing is complete. Second, in many regulating leg disk fragments new structures appear in a sequence that originates from the cut surface containing the blastema<sup>5,13</sup>. This same sequence

		A B C DEF
4	I	++-
1	П	+ + +
2	I	± +
2	п	+ + +
3	I	±++-
S	П	+ + +
4	I	- +++++
4	П	+ + +
-	I	- + ++ ++
5	п	+ + + +
6	I	- + + + (+)(+)
6	П	+ + +
~	I	++-++
1	п	+ + + +
8	I	+++++
0	П	+ + +

Fig. 4 Pairwise analysis of the regulatory potential of the presumed blastema area. 3/4L+EK fragments were cultured for 1 day in vivo; the implants were then cut into fragments I and II. I fragments were given an additional 6 days in adults before metamorphosis, whereas II fragments were injected into metamorphosing hosts. I and II fragments from the same 3/4L+EK implant were analysed in a pairwise manner. +, Sector present; -, sector absent; ++, sector present and duplicated. Sector considered present when two or more markers were identified. (+), One marker identified. Boxes indicate that the same structures were differentiated by both fragments. Underlined sectors indicate those clearly regenerated.

is followed by isolated blastemas. Figure 2a shows that I fragments cultured in adults for 6 days regenerated and/or duplicated sector B less frequently than sectors C and D. This sequence also is expressed in each case that regulates. For example, when sector B was regenerated, sector C was always present. Therefore, the information necessary for regulating, in a sequence, is present before the completion of wound healing.

Later in the regulative process, homotypic healing is completed and cells at the cut edge are confronted with new neighbours. We propose that blastema growth continues, and additional positional values are intercalated according to the shortest route, as expressed in the polar coordinate model. This proceeds until continuity is restored to the positional field.

Our study indicates that stimuli other than homotypic wound closure, such as heterotypic contacts or simply the presence of a free cut edge, could initiate regulation. We do not know what types of interactions initially determine whether a blastema will regenerate or duplicate. However, we hope our study stimulates new thoughts about how cells interact during pattern formation.

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### Estimates of dinosaur speeds from a new trackway site in Texas

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A method for estimating the speed of a dinosaurian trail-maker from the size of its footprints and stride has been described by Alexander1. However, when applied to known trackways, this method gave rather low speeds (1.0-3.6 m s<sup>-1</sup>). Russell and Béland<sup>2</sup> estimated a speed of 1.77 m s<sup>-1</sup> for a slowly moving ornithomimid and 7.54 m s<sup>-1</sup> for an allegedly rapidly running ornithopod. The latter estimate is questionable as there is some doubt concerning the number of prints (and thus the stride length) of the trail<sup>3,4</sup>. Dinosaurs responsible for trackways in British Columbia<sup>5</sup>, South Wales<sup>6</sup> and Queensland<sup>7</sup> all seem to have been moving slowly. Thus reliable estimates of dinosaur speeds are all rather low. Here I report dinosaur speeds based on trails at a new site from the Lower Cretaceous of Texas, some of which appear quite fast by Alexander's method1.

The site lies along Middle Copperas Creek in western Kimble County, Texas, on the F6 Ranch. The tracks occur in the lower third of an approximately 1-metre-thick, thin-bedded, very fine-grained limestone. Rock layers containing footprints are exposed in a narrow belt on either side of the creek bed. The limestone is heavily mud-cracked, and in some places ripple marks are preserved. The precise stratigraphical level of the locality is uncertain; the track-bearing unit is in either the lower Fort Terrett Formation or the upper Glen Rose Formation (US Gulf Coast age is Comanchean; European age equivalent of the relevant part of the Comanchean is Aptian-Albian).

All the F6 Ranch tracks seem to have been made by carnivorous dinosaurs (theropods) of various sizes. Trackway data are given in Table 1. A stride is taken as the distance between corresponding points on successive prints of the same foot; a pace is the distance between corresponding points on successive prints of the opposite feet, or half a stride. The larger the number of tracks in a trail, the more accurate will be the speed estimated from that trail. The estimated speeds of the F6 Ranch theropods range from 1.8 to 11.9 m s<sup>-1</sup>. Most of the speeds are within the range reported previously, but three are considerably faster (Fig. 1). Alexander indicated that mammals shift from walking to trotting or running when the ratio of stride/hip height reaches 2.0. On this basis, at least five of the F6 Ranch dinosaurs would have been moving with a faster gait than a walk, and the makers of trails 86 → 0-82, BLV → A3 and Q94 → Q98 were probably running.

Alexander et al.8 described a relationship between stride length, animal size and speed, based on observations of rapidly running African ungulates, that they felt to be more appropriate for fast gaits than Alexander's earlier equation. Applying this revised equation results in slightly greater speeds for the three

running dinosaurs (Table 1).

Russell9 suggested on anatomical grounds that ornithomimids could have reached speeds of 22.2 m s<sup>-1</sup>. Applying a formula taken from Bakker<sup>10</sup>, Coombs<sup>11</sup> calculated that some ornithomimids might have attained speeds of 26.9 m s<sup>-1</sup>, and that large theropods like Allosaurus and Tyrannosaurus might have had top running speeds of 12.8-14.7 m s<sup>-1</sup>. However, Coombs expressed doubts as to the reliability of these estimates. Nevertheless, if these estimates of top running speed in theropods are even approximately correct, they suggest that the estimated speeds of the F<sup>6</sup> Ranch running theropods are not unreasonable.

Although there is no obvious relationship between the sizes of the F6 Ranch dinosaurs and their speeds, only the smaller or medium-sized reptiles seem to have been running rapidly. One might expect moderate-sized dinosaurs to have reached greater speeds than their larger contemporaries 11,12, although this has

been disputed13

Coombs<sup>11</sup> concluded that the running abilities of dinosaurs were inferior to those of modern cursorial mammals, a contention that has been both supported12 and challenged 3 by other workers. Human athletes can achieve speeds of 10 m s short distances; greyhounds and horses reach speeds of 19 m s in races14. In the wild, African ungulates have been clocked at speeds of 7-14 m s<sup>-1</sup> (ref. 8), and ostriches may attain speeds of 12-14 m s<sup>-1</sup> (ref. 15). Thus the fastest of the F<sup>6</sup> Ranch theropods were moving at speeds greater than those reached by human athletes, but not as fast as the top speeds of modern cursorial animals. Whether the estimated speeds of the F6 Ranch running



Fig. 1 Three footprints of trail BLV → A3, made by one of the running dinosaurs. Pace lengths for this part of the trail are 271.8 cm (A1 → A2) and 261.6 cm (A2 → A3); stride length, 530.9 cm. Estimated speed of the dinosaur, 8.3 m s

Table 1 Estimates of dinosaur speeds at the F<sup>6</sup> Ranch site

		Mean print				
	No. of	length	Mean stride	Estimat	ed speed	Stride/hip
Trackway	prints	(cm)	(cm)	$(m s^{-1})$	(km h <sup>-1</sup> )	height
CHHB→G48	5	47	322.6	2.6	9.4	1.7
M-73 → M-66	6	45	299.4	2.5	8.8	1,7
9B→JOF9	3	42	337.8*	3.3	11.7	2.0
K-69 → K-70	2	42	284.4†	2.5	8.9	1.7
L-71 → L-72	2	41	287.0†	2.5	9.1	1.7
18D→PVW1	5	40	296.3	2.8	10.0	1.9
86 → 0-82	. 7	38	658.9	11.1 (11.9)‡	39.9	4.3
N-77 → MKR32	5	38	288.3	2.8	10.0	1.9
BLV→A3	4	37	538.5	8.3 (9.4)‡	29.9	3.7
E23 → HOPE	15	37	269.1	2.6	9.5	1.8
F-90 → P-92	4	37	260.3	2.5	8.9	1.8
H-52A → H-53	3	37	322.6*	3.4	12.4	2.1
J60 → J64	7	37	214.1	1.8	6.4	1.5
C-17→RW	3	34	259.1*	2.7	9.6	1.9
Q94→Q98	5	29	565.6	11.9 (12.1)‡	42.8	4.9

<sup>\*</sup> Estimate based on a single stride measurement.

theropods represent their top running speeds is, however, unknown.

I have assumed that the method of Alexander accurately estimates the speeds of dinosaurian trail-makers, but this can probably never be proved. However, the relatively long strides taken by three of the F<sup>6</sup> Ranch dinosaurs suggests that, unless they had unusually long legs, these reptiles were moving faster

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than the makers of any other known dinosaur trails, whatever their absolute speed.

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# Cytotoxic T lymphocytes induced by syngeneic mouse melanoma cells recognize human melanomas

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The recognition by T cells of antigens displayed or presented on the cell surface is generally restricted by the products of genes in the major histocompatibility complex (MHC)1. Thus, most cytotoxic T cells recognize antigens in conjunction with self MHC determinants, especially in the response against virusinfected lymphocytes2, chemically modified self antigens3 and tumour cells4. In these situations, the majority of cytotoxic T-cell clones seems to recognize MHC determinants unique to the individual alleles ('private'-type determinants). However, certain cell surface antigens seem not to be subject to such rigid MHC restriction<sup>5-7</sup>. These apparent exceptions could be incorporated into the MHC-restriction model by postulating the involvement of public-type determinants of MHC as restriction site, but until now no cross-killing across species barrier has been reported in these instances. We now report that mouse cytotoxic T cells induced in primary culture by syngeneic melanoma cells killed various human melanoma cell lines in an antigen-specific fashion.

Cytotoxic T lymphocytes (CTL) against syngeneic malignant melanoma cells (B16) were induced in in vitro primary culture of naive C57BL/6 mouse spleen cells with mitomycin C(MMC)treated B16 melanoma cells. The cytotoxic activity was melanoma specific, since the cytotoxic lymphocytes killed only syngeneic melanoma cells (B16) but not C57BL/6 lymphoma (EL-4), C57BL/10 sarcoma (S913) or A/J sarcoma (S1509a) cells. As shown in Fig. 1, the cytotoxic lymphocytes were able to lyse four different human melanoma cell lines (P-22, P-36, P-39, HMV-I) as well as mouse melanoma (B16), while they did not affect the other human cell lines tested—lymphoma (OKU) and cervix carcinoma (HeLa-S3). The cross-reactive cytotoxic activity was completely abrogated by treatment of the cells with monoclonal anti-Thy-1.2 and complement (C). This indicates that the cells responsible for the cross-reactive cytotoxicity have T-cell characteristics (Fig. 2). These results, therefore, clearly show that mouse CTL specific for syngeneic melanoma cells definitely cross-reacted with human melanoma cells, acting across a species barrier.

The cross-reactive specificity of the CTL was confirmed by using a cell inhibition assay, demonstrating that both human and mouse melanoma cells specifically inhibited CTL activity against syngeneic mouse melanoma cells (B16). In this experiment, the activity of the CTL was assayed against 51Cr-labelled B16 melanoma cells. Various unlabelled mouse and human tumour cell lines were added to the assay system as blockers with different cell numbers. The results clearly showed that both mouse and human melanoma cells (B16, P-22, P-36, P-39, HMV-I) but not other tumour cell lines (EL-4, OKU, HeLa-S3) gave strong inhibition of the CTL activity against B16 melanoma (Table 1). The inhibitory effects were specific, since the cytotoxic activity of the EL-4-specific CTL, induced in the in

<sup>†</sup> Estimate based on twice the value of a single pace. ‡ Revised formula for rapid gaits.

vitro primary culture of naive C57BL/6 spleen cells together with MMC-treated EL-4, was blocked by EL-4, but not by the mouse and human melanoma cells which inhibited melanoma-specific CTL (Table 1). Also, the possible involvement of the fetal calf serum component of the culture media as acquired determinants on the target cell surface was excluded, since the cytotoxic activity was observed on and inhibited only by melanoma cell lines from both human and mouse origins and not by other human and mouse tumour cell lines.

Thus our results show that malignant melanoma cells carry specific antigenic determinants shared with both mouse and human species—that is, melanoma-specific antigens have common antigenic moieties in different animal species. This is also supported by our finding that cytotoxic antisera raised in C57BL/6 mice hyperimmunized with MMC-treated B16 melanoma cells could specifically react with both mouse and human melanoma cell lines but not other cell lines (data not shown).

In general, the identity of the histocompatibility between T cells and their target cells is necessary for the cytotoxic T-cell

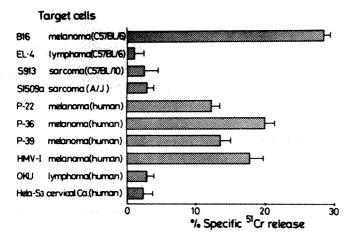


Fig. 1 Cross-reactive cytotoxic effect of mouse melanoma (B16)induced syngeneic (C57BL/6) killer cells on human melanomas. C57BL/6 cytotoxic lymphocytes (CTL) against syngeneic mouse melanoma (B16) cells were induced in the in vitro primary response. In brief,  $3 \times 10^7$  spleen cells from naive C57BL/6 mice were cultured alone as a control or with  $6 \times 10^5$  of MMC-treated B16 melanoma cells in 3 ml Dulbecco's modified minimal essential medium (DMEM) containing 5% fetal calf serum (FCS) and M 2-mercaptoethanol in 3.5 cm × 1 cm Petri dish (Falcon) at 37 °C in humidified 5% CO<sub>2</sub> in air. Five days later, the cells were pelleted by centrifugation and adjusted to  $6 \times 10^6$  viable nucleated cells per ml. To assay the activity of the cultured cells, the lymphoid cells  $(6\times10^5)$  were mixed with  $1.5\times10^4$  <sup>51</sup>Cr-labelled target cells (target/CTL ratio 1:40) in 0.2 ml of DMEM in a multi-well plate (NUNC) in quadruplicate. Target cells were mouse and human cell lines: C57BL/6 melanoma, B16; C57BL/6 lymphoma, El-4; C57BL/10 fibrosarcoma, S913; A/J fibrosarcoma, S1509a; human melanoma cell lines, SK-MEL-26(P-22), SK-MEL-28(P-36), MeWo(P-39), HMV-I; human lymphoma, OKU; and human carcinoma, HeLa-S3. The plates were incubated for 12 h at 37 °C and the radioactivity in a 0.1 ml supernatant from each well was counted by a well-type  $\gamma$  scintillation counter (Dainabot). Per cent lysis was expressed as percentage of specific  $^{51}$ Cr release calculated by the formula:

% Specific 51Cr release

$$= \frac{\text{c.p.m. experimental release} - \text{c.p.m. control release}}{\text{c.p.m. maximum release} - \text{c.p.m. control release}} \times 100$$

Control release was determined by incubating <sup>51</sup>Cr-labelled target cells with naive spleen cells of C57BL/6 mice cultured for 5 days without stimulator cells (B16). Maximum lysis was obtained by disrupting the target cells with saponin (Wako Pure Chemical). The bars indicate arithmetic means of the per cent specific lysis for four cultures ±s.d.

interaction1. In these studies, T cells have been shown to recognize MHC-encoded products, especially determinants that are highly polymorphic within species and those closely related to the serologically defined histocompatibility antigens. Thus, the requirement for T-cell recognition of MHC determinants has been demonstrated on the basis of MHC restriction of the cytotoxic activity. However, there are a few reports that MHC identity is not required for the interaction between cytotoxic T cells and allogeneic target cells<sup>8-10</sup>. For example, Burton et al.<sup>8</sup> have described the primary in vitro responses of murine cytotoxic T cells specific for oncofetal and plasmacytoma antigens that do not appear to be H-2 restricted. Similarly, Shaw et al. have demonstrated that the human cytotoxic T cell induced by trinitrophenol (TNP)-modified autologous cells was not restricted to lysis of TNP-modified human target cells without carrying shared HLA determinants with targets, and that the human CTL raised against TNP-self was clearly restricted with respect to lysis of mouse target cells. Nevertheless, these apparent exceptions could be explained by the MHC-restriction model that T cells recognize antigens in association with MHC determinants being widely shared within species.

However, the results presented have failed to show MHC restriction, and CTL specific for syngeneic mouse melanoma killed human melanoma cell lines across a species barrier. The products of genes in the I-E/I-C but not I-A subregion of the mouse H-2 complex share interspecies cross-reactive determinants with human HLA-DR antigens11,12, and human melanoma cell lines have also been reported to express HLA-DR like antigens 13,14. Thus the cross-reactive DR or Ia antigens are possible candidates to serve as 'self' determinants recognized by CTL. However, C57BL/6 mice as used in our studies have been shown not to express I-E/I-C subregion gene products<sup>11</sup>, so the B16 melanoma of C57BL/6 origin seems not to express cross-reactive Ia antigens on the cell surface. It is likely, therefore, that the CTL recognizes the melanomaspecific antigen itself, rather than an association of the antigen with MHC determinants. If so, the MHC restriction in the T-cell response may be determined by the nature of antigens with or without MHC products. If, however, common MHC determinants are conserved in different animal species (at least between mouse and man), it is also possible that mouse CTL recognizes melanoma-specific antigenic determinants in association with such conserved MHC determinants on human melanoma cells. We cannot yet determine whether one or more of these alternatives operate in tumour immunity, and experiments are under way to look into this question.

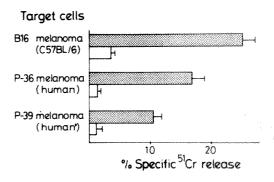


Fig. 2 Effect of the treatment with anti-Thy-1.2 on the cytotoxic activity of B16-induced syngeneic (C57BL/6) killer cells against mouse and human melanomas. The C57BL/6 CTL induced by syngeneic mouse melanoma cells (B16) as described in Fig. 1 was treated with a 1:1,000 dilution of monoclonal anti-Thy-1.2 (F7D5) for 30 min at 37 °C followed by incubation with well selected rabbit complement at 37 °C for 45 min. The activity of CTL treated was assayed by the same method as described in Fig. 1 legend. The bars indicate arithmetic means of the specific lysis of four cultures ±s.d. Solid and open bars indicate the activity of the CTL treated with complement alone or that treated with anti-Thy-1.2 and complement, respectively.

Table 1 Specific inhibitory effects of human melanoma cells on the cytotoxic activity of C57BL/6 killer T cells specific for B16 melanoma cells

		% Specific <sup>31</sup> Cr release			
Inhibitor cells	Labelled/unlabelled cell ratio	C57BL/6 anti-B16 on B16 melanoma	C57BL/6 anti-EL-4 on EL-4 lymphoma		
None	washin.	$23.9 \pm 1.2$	$34.3 \pm 2.2$		
B16 melanoma (C57BL/6)	1:9	$6.3 \pm 0.6$	$32.8 \pm 2.1$		
EL-4 lymphoma (C57BL/6)	1:9	$24.1 \pm 1.2$	$9.0 \pm 1.4$		
P-22 melanoma (human)	1:9	$3.2 \pm 1.2$	ND		
P-36 melanoma (human)	1:9	$5.0 \pm 1.4$	$26.6 \pm 2.1$		
P-39 melanoma (human)	1:9	$7.2 \pm 1.0$	$27.5 \pm 2.0$		
HMV-I melanoma (human)	1:9	$2.1 \pm 1.0$	ND		
OKU lymphoma (human)	1:9	$22.4 \pm 0.3$	ND		
HeLa-S3 carcinoma (human)	1:9	$20.4 \pm 0.9$	ND		

The activity of the C57BL/6 CTL-induced B16 melanoma or EL-4 lymphoma was assayed as described in Fig. 1 legend on 51Cr-labelled B16 or EL-4 tumour target cells (target/CTL ratio 1:40), respectively. Various unlabelled mouse and human tumour cell lines were added to the assay system as blockers with different cell numbers (labelled/unlabelled = 1:0, 1:1, 1:3, 1:9). The assay conditions and tumour cell lines were the same as described in Fig. 1 legend. The absolute number of the CTL and  $^{51}$ Cr-labelled B16 melanoma or EL-4 lymphoma cells as targets remained constant (target/CTL ratio of 1:40). Only blocker cell number was varied. Results are expressed as arithmetic means of the specific lysis of four cultures ±s.d.; ND, not determined.

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# **Antigen-driven helper** cell-independent cloned cytolytic T lymphocytes

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Cellular interactions between alloreactive cytolytic T lymphocytes (CTLs) and helper T cells have been suggested by the results of experiments in which lymphocyte subpopulations were defined by antisera against cell-surface markers' or by differential response to antigenic stimulation<sup>2,3</sup>. The development of T-cell cloning technology has allowed the isolation of alloreactive cloned helper and cytolytic cells, and the demonstration of collaboration between them in the generation of cytotoxicity<sup>4,5</sup>. Whereas some types of alloantigen-specific cloned T cells, including helper cells, proliferate in tissue culture in response to antigenic stimulation<sup>4-8</sup>, CTLs isolated thus far do not and require for proliferation the addition of exogenous T-cell growth factors (TCGFs) or helper cells which produce TCGF in response to antigenic stimulation<sup>4,5,7-10</sup>. We demonstrate here, at the clonal level, the existence of another type of CTL which proliferates in response to allogeneic stimulating cells without the overt addition of TCGF; it produces helperlike factor and thus can be characterized as a helper cellindependent CTL.

T-cell clones were derived by limiting dilution from 5-day C57BL/6 (B6) anti-DBA/2 (DBA) bulk mixed lymphocyte culture (MLC) populations as previously described<sup>7</sup>. Limiting dilution microcultures for cloning were seeded on day 5 of MLC with  $0.25\,\mathrm{B}6$  anti-DBA cells per well,  $1\times10^6$  irradiated (2,000 rad) DBA spleen cells and 30% (v/v) secondary MLC supernatant (2° MLC SN)11 as a source of growth factors, including TCGF. After 7-8 days, growing clones were further expanded numerically in larger culture vessels in the presence of DBA spleen cells and 20% 2° MLC SN and maintained by subculturing at 5-7-day intervals. Such cloned populations were tested repeatedly for cytolytic activity against P815 (DBA origin) and EL4 (B6 origin) tumour target cells in both direct and lectin-dependent 51Cr release assays and for their ability to proliferate in response to allogeneic DBA stimulating cells.

Table 1 shows the proliferative responses of two cloned CTL populations and two cloned helper populations. Clones 17-11 and 17-4 are CTLs which cause 50% <sup>51</sup>Cr release from P815 target cells in a direct test at effector/target ratios of 1:1 to 3:1; no detectable lysis of EL4 cells is observed at ratios of up to 30:1. Clones 12-11 and 17-10 can be characterized as noncytolytic cells based on their inability to kill P815 or EL4, even in the presence of lectin, and as helper cells based on their ability to produce soluble factor(s) capable of stimulating proliferation in a TCGF assay using long-term (days 14-18), resting, primary MLC populations as indicator cells (unpublished observation).

All clones proliferated in culture when 2° MLC SN was present, regardless of whether splenic filler cells were also present (Table 1). However, in the absence of exogenous 2° MLC SN, only certain clones, such as helper cell clones 12-11 and 17-10, proliferated in response to specific allogeneic DBA stimulating cells. Cultures of CTL clone 17-11 and allogeneic DBA stimulating cells did not incorporate amounts of <sup>3</sup>Hthymidine (TdR, 3,066 c.p.m.) greater than those incorporated in control cultures using syngeneic B6 stimulators (1,048 c.p.m.) or stimulating cells alone (3,736 c.p.m.) (Table 1). This CTL clone thus exhibits the growth requirement for TCGF typical of CTL clones previously described<sup>4,5,7-10</sup>; in our experience, six B6 anti-DBA CTL clones behaved in the proliferative assay like CTL clone 17-11. In contrast, CTL clone 17-4 demonstrates the distinct ability to proliferate in response to allogeneic DBA stimulating cells, even in the absence of 2° MLC SN (23,792 c.p.m.).

Table 1 Proliferative response of T-cell clones

24.97			Stimulating cells	
Responding cell clone	2° MLC SN	None	C57BL/6 <sub>x</sub>	DBA/2 <sub>x</sub>
17-11	 +	$490 \pm 240$ * $11,808 \pm 776$	$1,048 \pm 242$ $10,194 \pm 460$	$3,066 \pm 278$ $41,628 \pm 3,566$
17-4	 +	$1,084 \pm 300 \\ 80,432 \pm 5,380$	$1,348 \pm 124$ $64,296 \pm 912$	$23,792 \pm 298$ $48,270 \pm 3,816$
12-11	- +	$1,764 \pm 1,348 \\ 28,914 \pm 2,614$	$2,554 \pm 1,090$ $30,424 \pm 1,864$	$42,200 \pm 958$ $39,660 \pm 1,032$
17-10	<del></del>	$904 \pm 608$ $21,660 \pm 2,200$	$1,512 \pm 406$ $14,194 \pm 376$	$18,192 \pm 602 \\ 25,174 \pm 1,598$
None	. 4 Hawari — <mark>—</mark>	NT NT	$1,346 \pm 888$ $1,264 \pm 146$	$3,736 \pm 2,482$ $3,690 \pm 130$

Proliferative response of alloreactive clones in MLC. Clones were derived by limiting dilution from 5-day B6 anti-DBA bulk MLC populations. Cloned cells were expanded and maintained by subculturing  $1-5\times10^4$  cloned cells at 5-7-day intervals in 16-mm Costar wells containing 20% v/v 2% MLC SN and  $6\times10^6$  irradiated (2,000 rad) DBA spleen cells. The culture medium consisted of Dulbecco's modified Eagle's medium supplemented with 2% fetal bovine serum and additional amino acids. For the proliferative assay,  $1\times10^4$  cloned cells obtained 5-7 days after previous subculture were cultured (0.2 ml final volume) in flat-bottom microtitre plates either alone or with  $1\times10^6$  irradiated syngencic B6 or allogencic DBA spleen cells in the presence or absence of 25% 2° MLC SN. Cultures were pulsed on day 2 for 7 h with 2  $\mu$ Ci  $^3$ H-TdR. Cells were collected on to glass fibre filters and  $^3$ H-TdR incorporation was determined by liquid scintillation spectrometry. Results are given as mean c.p.m.  $^3$ H-TdR incorporated  $\pm$  s.d. of triplicate cultures. NT, not tested.

Because cells of putative clone 17-4 exhibited the lytic characteristics of CTLs and the proliferative characteristics of T-helper cells, we investigated whether both functions were in fact mediated by the progeny of a single cell, rather than two functionally distinct cells contained within the 17-4 population, by analysing subclones derived from 17-4 cells in both the cytolytic and proliferative assays. Subcloning was accomplished by plating 17-4 cells in limiting dilution microcultures at a concentration of 0.1 cells per well. Of 168 microcultures established, 20 were observed to contain growing cells when examined microscopically 7 days after culture initiation. Thus, cells from putative clone 17-4 had 100% plating efficiency (16 positive microcultures were expected based on the Poisson distribution). Cells from five positive subcloning cultures were picked, expanded numerically and tested for both cytolytic activity against P815 and proliferation in response to irradiated DBA spleen cells. Figure 1 demonstrates that cells of parent clone 17-4 and all five tested subclones were highly cytolytic towards P815 target cells, causing 50% <sup>51</sup>Cr release at effector/target ratios between 1.8:1 and 3:1. In addition, cells of all five 17-4 subclones were, like those of the 17-4 parent clone, able to proliferate in response to irradiated DBA spleen cells in the absence of overtly added 2° MLC SN (Table 2). The probability that any one of these subclones could have been derived by chance alone from more than one cell in the parental population of putative clone 17-4, given the 0.1 cell per well dilution used for subcloning, is  $5 \times 10^{-2}$ ; the probability for all five subclones is  $3 \times 10^{-7}$ . Thus, there can be no reasonable doubt that both cytolytic activity and proliferative response to allogeneic cells are in this case functions of the progeny of a single T cell. Karyotypic analysis of 17-4 cells has shown them to have the normal chromosome number of 2n = 40.

The proliferative response of cells of clone 17-4 in the presence of allogeneic stimulating cells could conceivably be induced by factors secreted by cells, presumably T cells, within the irradiated stimulating cell population, which recognize the cloned cells as foreign—that is, by 'back stimulation'. However, the data in Table 3 demonstrate that allogeneic cells depleted of T cells by pretreatment with anti-Thy-1.2 antibody and complement stimulate cells of subclone 17-4.24 to proliferate,

Table 2 Proliferative response of clone 17-4 and its subclones

Responding cell clone	2° MLC SN	None	Stimulating cells	
	2° MLC SN	None		
			$C57BL/6_x$	$DBA/2_{x}$
17-4	<del>-</del>	$1,038 \pm 563$ * $34,303 \pm 1,446$	$1,628 \pm 428$ $19,932 \pm 319$	$24,178 \pm 800$ $21,079 \pm 1,920$
17-4.21	<del>-</del>	$981 \pm 505$ $72,557 \pm 5,457$	$2,136 \pm 361$ $45,103 \pm 7,873$	$32,506 \pm 1,109$ $34,318 \pm 1,530$
17-4.22	+	$616 \pm 306$ $23,316 \pm 1,256$	$638 \pm 18$ $19,998 \pm 915$	$12,220 \pm 483$ $26,654 \pm 577$
17-4.23	<del>-</del> +	$2,866 \pm 902$ $61,109 \pm 3,340$	$1,734 \pm 574$ $36,086 \pm 2,019$	$20,315 \pm 2,689$ $30,353 \pm 1,049$
17-4.24	<del></del> +	$439 \pm 294$ $83,044 \pm 814$	$1,297 \pm 1,025$ $40,846 \pm 3,232$	$42,153 \pm 3,249$ $40,634 \pm 5,706$
17-4.28	<del>-</del>	$309 \pm 278$ $48,593 \pm 1,819$	$898 \pm 332$ $31,956 \pm 3,678$	$14,102 \pm 2,614$ $41,366 \pm 1,003$
None	<del>-</del> +	NT NT	$1,152 \pm 641$ $1,274 \pm 394$	$1,328 \pm 428 \\ 2,752 \pm 799$

Proliferative response in MLC of CTL clone 17-4 and its subclones. Subclones were derived by plating 17-4 cells at a concentration of 0.1 cells per well in the presence of  $1 \times 10^6$  irradiated DBA spleen cells and 30% 2° MLC SN. Cloned cells were expanded, maintained and tested for proliferative responses in MLC as described in Table 1 legend.

Table 3 Proliferative response of cloned cells to T-cell-depleted stimulating cells

Responding	Treatment of stimulating	Stimulating cells			
cell clone	cell population	None	C57BL/6 <sub>x</sub>	DBA/2 <sub>x</sub>	
17-4.24	C'	276±80*	$796 \pm 300$	$4,505 \pm 548$	
	Anti-Thy-1.2+C'	270200	$548 \pm 262$	$6,215 \pm 330$	
None	C' Anti-Thy-1.2 + C'	NT	$561 \pm 125$ $288 \pm 35$	$553 \pm 102$ $505 \pm 71$	

Proliferative response of subclone 17-4.24 to T cell-depleted stimulating cells. Cloned cells were tested for proliferation in response to allogeneic spieen cells in the absence of 2° MLC SN as indicated in Table 1. Syngeneic B6 or allogeneic DBA spleen cells were incubated at room temperature for 30 min with a 1:1,000 dilution of monoclonal anti-Thy-1.2 antibody (NEN), followed by incubation at 37 °C for 45 min with a 1:10 dilution of rabbit complement (C'). Control aliquots were incubated with complement alone. Antibody and control-treated cells were washed three times, irradiated (2,000 rad) and used as stimulating cells ( $1 \times 10^6$ cells per culture) in MLC with cloned responding cells.

thus eliminating back stimulation as the sole or primary explanation for the proliferative response. In addition, specificity analyses using congenic H-2 recombinant strains have shown specificity of clone 17-4 in both the proliferative and cytotoxic assays against D<sup>d</sup>-encoded antigens; no proliferative response (and thus no back stimulation that might be expected) is observed even when cells from strains bearing entirely different H-2 antigens (H-2<sup>s</sup> and H-2<sup>k</sup>) are used as potential stimulators of 17-4 cells.

We have shown here, on the clonal level, that alloreactive CTLs can be divided into at least two distinct categories based on their requirements for proliferation. The first is the 'conventional' CTL represented by our clone 17-11 and also isolated by others<sup>4,5,7-9</sup>. We present evidence here for the existence of a second type of CTL, exemplified by clone 17-4. In contrast to cells of the first category, this cell does not require independently derived sources of helper factor for proliferation, as shown by its ability to proliferate in response to stimulating cells bearing the sensitizing alloantigens. In this respect clone 17-4 is a helper cell-independent CTL clone, and represents, to our knowledge, the first proven clone with such characteristics to be reported.

We emphasize that clone 17-4 is the only CTL clone of the seven we have tested to date which exhibits responsiveness in MLC to allogeneic cells. Thus, the frequency of such cells in bulk MLC populations from which the clones are derived (or the frequency of detection) seems to be relatively low. However, the existence of this type of cell has recently been confirmed both independently (A. Glasebrook, personal communication) and in our own laboratory (D. Roopenian, unpublished observation).

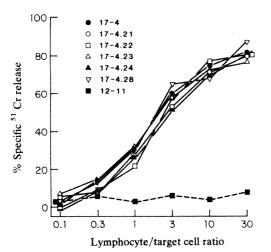


Fig. 1 Cytolytic activity of clone 17-4 and its subclones. Cells from clone 17-4 and five subclones (solid lines) and helper cell clone 12-11 (dashed line) were tested at the indicated effector cell/target cell ratios for cytolytic activity against  $2\times10^3$   $^{51}$ Cr-labelled P815 target cells in a 3.5-h assay<sup>14</sup>. Cells used for cytotoxicity testing were obtained 5 days after subculture.

Glasebrook and Fitch have reported that helper cell clones can utilize for proliferation growth factor(s) which they themselves produce12. Preliminary evidence suggests that cells of CTL clone 17-4, like cloned helper cells, secrete a soluble factor when stimulated with allogeneic cells which induces proliferation and cytolytic activity in resting, long-term (day 14) MLC populations. Whether the factor produced by clone 17-4 is the same as that (those) produced by the conventional type of non-cytolytic helper cell is not known, but such a factor could be involved with antigen in the induction of proliferation by the clone. We thus propose that clone 17-4 has both helper and CTL functions previously considered unique to different T-cell subsets. Such attributes would presumably constitute an effector T cell that depends only on antigen for expansion. Whether this type of cell represents a stage in the T-cell lineage before or after differentiation of cells exhibiting mutually exclusive functions remains unknown.

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## Inward current channels activated by intracellular Ca in cultured cardiac cells

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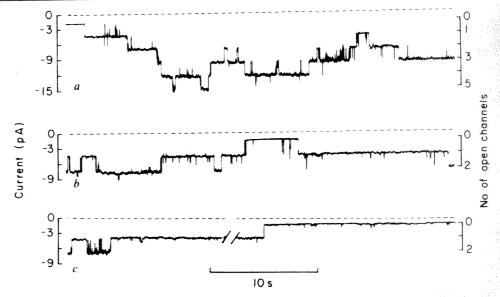
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Present concepts of excitable membrane function are based primarily on knowledge of two classes of channels: those activated by neurotransmitters<sup>1</sup> and those activated by membrane potential2. Recent evidence suggests that these notions may have to be modified to include other channel types, such as special ion channels activated by membrane potential but regulated by ligands<sup>3-5</sup>. We report here studies on single channel currents recorded from heart muscle cells, in which we have found a channel, abundant in cardiac membrane, which does not seem to belong in any of the familiar categories. This channel shows little selectivity between cations, but excludes anions. It is activated by intracellular Ca ions but is not appreciably affected by membrane potential.

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Fig. 1 Recordings of single-ion currents at three channel from inside-out concentrations membrane patch of cultured (day 4) rat ventricular muscle cell at 26 °C. pipette (external side contained Na-saline membrane) with 6 µM Ca. The broken line above each trace marks zero current; inward current is shown as a downward deflection. The membrane patch was voltage-clamped to -70 mV, which is seen to require voltage-clamped to ~1.5 pA (patch resistance ~50  $G\Omega$ ) when all channels are closed. The unit current is ~2.7 pA (conductance 38 pS; in b and c two smaller inward current steps are clearly a, Bathing solution (cytoplasmic side of membrane) of Na-saline with 6 µM Ca (recording started soon after patch isolation). b, Bathing solution of K-saline with 1.5 µM Ca (flow started 11 s before



start of record). c, Bathing solution of 'Ca-free' saline + EGTA (10  $\mu$ M). Flow started a few seconds before start of record. After brief flickering, one channel remained open for 35 s before closing (the break in the record denotes a 22-s gap throughout which this channel alone remained open); then all channels remained closed while EGTA was still present in the solution. Re-addition of calcium (not shown) caused the channels to open again. Note the quiet baseline in a and b when all channels were closed and the increase in 'noise' when the channels were open.

In our experiments we used myocytes obtained from neonatal rat hearts and cultured by a method similar to that described elsewhere<sup>6</sup>. The cells were exposed to trypsin (0.025%) for 24 h before seeding on day 0 and used before day 7. We selected for our experiments single cells having spindle-like shapes and striations. Single-channel currents were recorded from isolated patches of membrane with either the cytoplasmic surface (inside-out patch) or the external surface (outside-out patch) facing the bathing solution<sup>7</sup>. The Na-saline solution contained (mM): NaCl, 137; KCl, 5.4; MgCl<sub>2</sub>, 1.05; HEPES buffer, 5.0; glucose, 10; pH 7.3. In the K-saline solution, NaCl was replaced by KCl. CaCl<sub>2</sub> was added, and the final Ca concentration determined by atomic absorption spectroscopy.

Channel currents of the type shown in Fig. 1 were seen in most isolated membrane patches. We have interpreted such records as arising from the superimposed currents of several independent channels that can only open and close, but we cannot exclude the possibility that individual channels have multiple equally spaced conductance levels. The rate of channel opening was clearly dependent on the Ca concentration on the cytoplasmic side of the membrane (see Fig. 1). Considerable channel activity was usually observed in the presence of 6 µM Ca (Fig. 1a). After reduction of the Ca concentration to 1 or 1.5 µM, the activity decreased (Fig. 1b) but was still substantial. In the presence of 'Ca-free' saline + EGTA (10 µM), all channels eventually closed (Fig. 1c). In several experiments it was possible to change from Ca-containing solution to EGTA-saline and back several times, and to observe repeatedly the disappearance and reappearance of the single-channel currents. The assessment of the Ca-concentration dependence of channel opening was complicated by the fact that even when the Ca concentration was kept constant, the channel opening rate was usually observed to decline over a period of several minutes. A subsequent increase in activity was often seen when the Ca concentration was raised (for example, to 20 or 50 µM). However, changes in Ca concentrations over a wide range did not affect single-channel conductance. In many experiments similar channel currents were observed when the patch clamp pipette was applied to the intact cell, without removal of the membrane patch; in these cases no obvious contracture of the cell was observed.

The opening and closing of the Ca-dependent channels did not show any strong dependence on membrane potential at a Ca concentration of  $20~\mu M$ . Single-channel currents occurred with

similar frequencies at membrane potentials from -140 mV to +70 mV. In other experiments, records were obtained while stepping the membrane potential either from -70 mV to +70 mV for 100 ms, or from -70 or -120 mV to -30 mV for 5 s. When a series of such records is averaged, if the number of open channels were voltage-dependent it would be expected that the mean current would show a relaxation towards a new equilibrium value following a step in membrane potential. Such relaxations were observed, but they were very small, indicating that the open-close equilibrium changes less than e-fold for 200 mV. We cannot exclude the possibility that stronger voltage dependence might be present in other conditions.

The opening and closing patterns of these channels are rather complex. In conditions of low activity when at most one channel

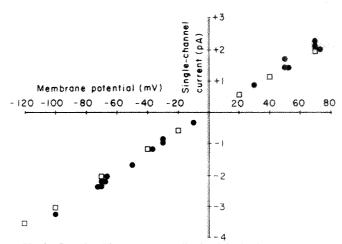


Fig. 2 Relationship between amplitude of single-channel current and membrane potential, with symmetrical and asymmetrical solutions at 25-26 °C. Values at positive potentials were obtained by short (100 or 200 ms) jumps from the holding potential (-70 mV) to a positive potential. All measurements were made at 10-mV intervals, but, when necessary for clarity, the symbols have been slightly offset horizontally. ♠, Na-saline with 20 μM Ca on both sides of the membrane; values from five different cells (insideout membrane patches) on day 6 of culture. □, K-saline with 1 μM Ca on cytoplasmic side of membrane, and Na-saline with 2 mM Ca on the external side of the membrane (outside-out membrane patch); cells were used on day 2 of culture.

was open, the mean open lifetime was about equal to its s.d. with values of ~100-300 ms. The shorter openings and poorly resolved spikes (see Fig. 1) represent channel lifetimes of this order. In most cases there were much longer channel openings of many seconds duration (Fig. 1). Often, for example, one channel appeared to be 'permanently' open and the resulting current formed a baseline on which the faster activity described above was superimposed. One channel seemed to remain open for ~35 s after addition of EGTA, before it eventually closed (see Fig. 1c). Even in patches where we had evidence for the presence of multiple channels, long periods occurred when one of the channels opened with a high probability and the other channels appeared not to function. This suggests that channels can also enter a long-lived closed state.

Conductance through the ion channel was approximately ohmic, as shown in Fig. 2 where the single-channel conductance was ~30 pS at 25 °C. Typically, single-channel conductances in over 50 patches ranged between 30 and 40 pS at 25-27 °C. Figure 2 shows that with identical saline on both sides of the membrane, the reversal potential for the channel current is close to the expected value of 0 mV. When all NaCl was replaced by KCl on the cytoplasmic surface (Fig. 2, □) the reversal potential was unchanged. Thus the ion channel must have similar permeabilities to Na and K ions. In another experiment we applied a salt gradient across the membrane by diluting the pipette saline to 1/5 with distilled water. Single-channel currents recorded in this condition reversed sign at -40 mV, as expected for cation selectivity. In addition, when chloride was replaced by the presumably less permanent anion, aspartate, the reversal potential did not change. These results indicate negligible anion permeability of the ion channels. The temperature dependence of the unit current (Fig. 3) has a  $Q_{10}$  of  $\sim 2.3$  (two experiments), which is unusually large; for example, a  $Q_{10}$  of 1.2-1.3 has been reported for ACh-activated channels<sup>8</sup>

In addition to the predominant channels described above, various other channel types were seen, including a number of small inward channel currents present at -70 mV (see Fig. 1b, c) and occasional Ca-dependent inward currents that were unusually large (50-60 pS). Voltage-dependent Na, Ca and K channels were also observed.

The ion channels described here resemble ACh-activated endplate channels in their conductance, and in their poor discrimination between Na and K ions, as well as in their low permeability to Cl ions<sup>10</sup> but their conductances are more temperature sensitive, have much longer open lifetimes, are less voltage dependent and are unaffected by carbachol. They resemble Ca-activated K channels 11,12 in that they are activated by Ca on the cytoplasmic side of the membrane, and in their

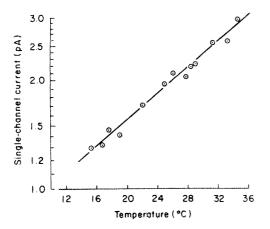


Fig. 3 Temperature dependence of the amplitude of the singlechannel current. The ordinate is a logarithmic scale. Cells were used on day 2. Inside-out membrane patch with K-saline + 20 µM Ca in the pipette and Na-saline + 20 µM Ca in the bathing solution; membrane potential -70 mV.

complex kinetics. However, they seem to require higher concentrations of Ca for activation, are not so voltage-dependent and lack the cation selectivity of Ca-activated K channels.

Several authors<sup>13-15</sup> have described oscillatory inward currents that occur in cardiac Purkinje fibres after depolarizing clamp steps during ouabain exposure or with high extracellular Ca concentration. This transient inward current has many features in common with the ion channels described here. It is activated by intracellular Ca ions and not by voltage directly and has a reversal potential around -5 mV indicating poor selectivity between Na and K ions<sup>14</sup>. Kass et al. <sup>14</sup> speculate that the underlying mechanism for the transient inward current could be non-selective 'leak' channels which, in normal conditions, would provide the background current required for pacemaking activity in cardiac cells. This hypothesis would be consistent with our results.

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# Free Ca<sup>2+</sup> increases in exponential phases during mouse oocyte activation

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A dramatic rise in cytoplasmic free Ca2+ concentration has been shown to occur during fertilization and artificial activation in the oocytes of both the medaka fish1 and sea urchin2. Indirect evidence has implicated Ca2+ in the parthenogenetic activation of mammalian oocytes. Mouse oocytes can be activated by the intracellular injection of Ca2+ but not Mg2+ (ref. 3), and hamster oocytes by exposure to the calcium ionophore, A23187 (ref. 4). We report here measurements of cytoplasmic free Ca2+ during the artificial activation and fertilization of single mouse oocytes injected with the Ca2+-sensitive photoprotein aequorin. Free Ca2+ rises exponentially from a resting level of below 0.1 μM to >5  $\mu M$  over a period of 10-30 min. A series of oscillatory Ca<sup>2+</sup> transients precedes this Ca<sup>2+</sup> rise during fertilization, but not during artificial activation.

The light from a single 'resting', unfertilized oocyte (70 µm diameter, 200 pl volume), injected with aequorin to  $\sim 5\%$  of its volume, ranged typically between 1 and 16 photoelectrons per s above a background of  $14 \, \text{s}^{-1}$  (Fig. 1A, B). The light signal was detected by holding the oocyte in a 75-µl-capacity reflecting cup positioned just below the photocathode of a low-noise photo-

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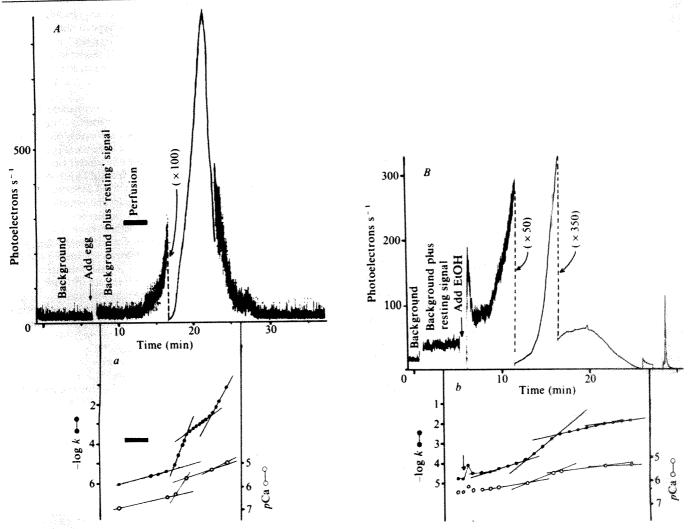


Fig. 1 A, a, Effect of an activating concentration of benzyl alcohol (0.5% v/v) on an aequorin-injected oocyte. A, Chart recording of the aequorin signal, expressed as photoelectrons s<sup>-1</sup> against time. The resting signal of 6 photoelectrons s<sup>-1</sup> remained constant until medium containing 0.5% benzyl alcohol was superfused (solid bar; the egg was exposed to benzyl alcohol up to the end of the experiment). Immediately the signal rose rapidly. The sensitivity of the recorder was reduced by a factor of 100 between 17 and 23 min. The signal returned to background level as the aequorin was consumed. In some experiments total consumption of aequorin was confirmed by adding SDS or distilled water to lyse the oocyte. a, The same data transformed to  $-\log k$  ( ) against time, where k is the fraction of acquorin consumed per s (that is, the ratio of the light signal in photoelectrons  $s^{-1}$  to the total number of photoelectrons yet to come from the active aequorin remaining in the oocyte). Note the piece-wise linearity of this plot, which implies that [Ca2+], increases in exponential phases. The plot is also shown in terms of pCa units (O). B, b, Effect of an activating concentration of ethanol (8.6% v/v) on an aequorin-injected oocyte. B, Chart recording of the aequorin signal in photoelectrons s<sup>-1</sup> against time. Recorder sensitivity was reduced 50-fold and 350-fold at 12 and 17 min. Ten microlitres of medium with the acquority signal in photoelectrons against time. Recorder sensitivity was reducted 50-load and 25-load at 25-load and 12-load and photoelectrons against time. Calibration measurements were made in vitro at 35 °C with EGTA Ca<sup>2+</sup> buffers containing (mM) KCl, 150; PIPES, 10; EGTA, 10; free Mg<sup>2+</sup>, 5 at pH 7.4. The stability constants of Ca–EGTA and Mg–EGTA, corrected for 37 °C and pH 7.4, were calculated at  $10^{7.44}$  and  $10^{2.46}$  M<sup>-1</sup> respectively 14. Our calibration curve of  $-\log k$  against pCa is linear (with slope 3.0) from  $-\log k = 5.5$  to 2.0. (Benzyl alcohol and ethanol have a direct effect on the emission of light by acquorin, as with other anaesthetics 15. Benzyl alcohol (0.5%) raised k by <0.1 log unit, but ethanol (8%) raised k by 0.8 log unit (P.H.C., unpublished results). This accounts for the initial jump in log k seen in the ethanol experiment (arrow). Quantum yield is not affected.) Occytes were collected from superovulated random-bred albino females (MF1; Olac, Bicester) and maintained at 37 °C in a Krebs-Ringer's bicarbonate-buffered medium (No. 16; ref. 16) gassed with 5% CO<sub>2</sub>, 95% air. Cumulus cells were removed with hyaluronidase in medium 16. Oocytes for the fertilization experiments were treated, after injection, with 0.03% α-chymotrypsin (type II; Sigma) to remove the zona pellucida. Oocytes were injected at room temperature in medium M2 (ref. 3), which is medium 16 modified by partly replacing the bicarbonate buffer with HEPES buffer. The aequorin was dialysed against an injection buffer containing 30, 80 or 150 mM KCl, 10 μM 3-(N-morpholino)propanesulphonic acid (MOPS) and 25 μM EDTA, pH 9.0. Micropipettes with a tip diameter of -2 m were pressure-filled (at 7 bar) through the tip5 with the acquorin solution, which was injected by pulses of gas pressure (up to 2 bar) into an oocyte held on a siliconized heat-polished pipette. The oocyte was contained in a minimal bubble of medium at the end of the holding pipette, surrounded by silicone oil (M100; Bayer, Leverkusen). The aequorin was thus protected from the Ca<sup>2+</sup> in the medium before penetration of the oocyte. All Ca<sup>2+</sup> measurements described were done in medium 16 at 35 °C, except for the two experiments shown here, in which medium M2 was used. Oocytes were between 15 and 22 h post-HCG (human chorionic gonadotropin) at the time of the experiments.

multiplier. Detection limits proved to be better than predicted. Assuming homogeneous Ca<sup>2+</sup> distribution, and assuming that the conditions in vivo can be sufficiently matched in vitro, the rate of aequorin consumption, k, can be calibrated<sup>6,7</sup> for a given  $Mg^{2+}$  concentration, to give the free intracellular  $Ca^{2+}$  concentration,  $[Ca^{2+}]_i$ . The mean value of k measured in 21 'resting' oocytes at 35 °C corresponds to values for  $[Ca^{2+}]_i$  of  $5\pm1\times10^{-8}$  M if  $[Mg^{2+}]_i$  is 5 mM, and  $1.3\pm0.1\times10^{-7}$  M if  $[Mg^{2+}]_i$  is 10 mM. For 10 of the oocytes,  $\log k$  was less than the value for  $Ca^{2+}$ -independent light emission with 5 mM  $Mg^{2+}$  ( $\log k = -6.25$ ), which suggests that, for these oocytes at least,  $[Mg^{2+}]_i$  was greater than 5 mM. If  $[Mg^{2+}]_i$  can vary significantly between

oocytes, then the resting  $[Ca^{2+}]_i$  could be substantially lower than  $1 \times 10^{-7}$  M.

Brief treatment (5–10 min) of mouse oocytes with benzyl alcohol (a local anaesthetic) or ethanol induces activation; that is, meiosis II proceeds and pronuclei form, and over 70% of the activated oocytes can develop into blastocysts when cultured for a further 4 days<sup>8</sup>. On exposure of aequorin-injected oocytes to medium containing an activating concentration of benzyl alcohol (0.3–0.5%) or ethanol (5.0–8.6%),  $[Ca^{2+}]_i$  rose to  $5 \times 10^{-6}$  M (Fig. 1). A slightly lower concentration of benzyl alcohol (0.2%) did not induce this dramatic change in  $[Ca^{2+}]_i$ , although a small increase in signal was sometimes detected. Similarly, large

increases in  $[Ca^{2+}]_i$  were seen, after delays of up to 1 h, in oocytes held in Ca-free medium (2/3 oocytes), Ca-free medium with 1 mM Sr<sup>2+</sup> added (2/3 oocytes, Fig. 3C) and normal Ca-containing medium without any experimental intervention (11/54 oocytes). The observation of the  $Ca^{2+}$  change in oocytes in Ca-free medium explains the apparent paradox that both the injection of  $Ca^{2+}$  (ref. 3) and exposure to Ca-free medium<sup>9,10</sup> activate oocytes.  $Ca^{2+}$  rose to  $5\times 10^{-6}$  M in oocytes in Ca-free medium with the same time course as spontaneously activating oocytes in medium containing 1.71 mM Ca, suggesting that the increase in  $[Ca^{2+}]_i$  is due to the release of  $Ca^{2+}$  from internal stores.  $Sr^{2+}$  enhances activation in Ca-free medium<sup>10</sup>, but does not alter the form of the  $Ca^{2+}$  release.

To compare the [Ca2+] responses during artificial activation with the response during fertilization, single zona-free oocytes containing aequorin were placed with sperm under the photomultiplier. In three oocytes, a series of 8 to 12 Ca2+ transients was observed after delays of between 5 and 90 min (Fig. 2). These transients occurred over a period of 45 min in two of the oocytes and 90 min in the third, and preceded a large [Ca<sup>2+</sup>], rise similar to that seen during artificial activation (Fig. 3A, B). The first two or three transients had 4-6 oscillations of large amplitude. The rapid rise and fall of the transients and the oscillations within each transient are in contrast to the slow rise during artificial activation, and the base level between transients starts to rise towards the end of the series in the first phase of the 'activating' rise in [Ca<sup>2+</sup>]<sub>i</sub> (data not shown). This suggests that the signal during the transients does not arise from the entire oocyte, but rather from a localized region, which may be at the site of sperm-egg fusion or near to the plasma membrane of the oocyte. In the medaka fish oocyte (diameter ~1 mm) the [Ca<sup>2+</sup>], increase starts at the site of sperm-egg fusion and then spreads around the cell1. Each series of transients forms a coherent pattern. Thus we believe that a single sperm can initiate a series of transients; these were never seen when oocytes were

activated in the absence of sperm (35 oocytes). In addition, a single fertilizing sperm induces recurring hyperpolarizations in hamster oocytes<sup>11</sup>, which could be caused by cyclic increases in  $[Ca^{2+}]_i$  corresponding to the  $Ca^{2+}$  transients reported here. It is therefore likely that the series of  $Ca^{2+}$  transients were induced by fertilizing sperm, although it was not possible to confirm that sperm had penetrated the oocytes.

The rise in [Ca<sup>2+</sup>], during activation is piece-wise exponential; that is,  $pCa \left(-\log \left[Ca^{2+}\right]_{i}\right)$  increases linearly with time within successive phases (Figs 1a, b, and 3). In some cases four phases were discernible, giving the appearance of a zigzag (Figs 1, 3C). In one case (see Fig. 3B) there was just one phase. Linearity is seen, to within observational accuracy, from pCa = 6.4 to 5.4, that is, during a 10-fold increase in  $[Ca^{2+}]_i$ . Linearity of  $\log k$ within phases was consistently observed. The simplest interpretation is that [Ca<sup>2+</sup>], rose uniformly throughout the whole cytoplasm, in which case the rise within each phase was exponential, and therefore the rate of Ca2+ increase was proportional to [Ca<sup>2+</sup>]<sub>i</sub>. If [Ca<sup>2+</sup>]<sub>i</sub> is not uniform, the interpretation of k becomes complex, particularly as k is a nonlinear function of  $[Ca^{2+}]$ , and a model to account for linearity in  $\log k$  would have to include the diffusion of aequorin as well as Ca2+. While the light signal reached a peak and then dropped rapidly  $\log k$ was often still rising linearly (for example, as in Fig. 1a). The fall in light signal was due to the consumption of aequorin. In this circumstance the dependence of the rate of Ca2+ increase on  $[Ca^{2+}]_i$  necessary to account for the linearity in  $\log k$  would become highly contrived if it were assumed that [Ca<sup>2+</sup>], was significantly non-uniform. Step changes in the slope of the graph of pCa could be generated by step changes in the Ca2+ release parameter (the ratio of the net rate of Ca2+ release to [Ca2+], denoted by  $R_{Ca}$ ). A biochemical system such as that proposed for biochemical oscillators<sup>12</sup> might determine the value of  $R_{Ca}$ . Alternation between stable states of this system might cause step changes in  $R_{Ca}$ . A step increase in  $R_{Ca}$  which raises the rate of

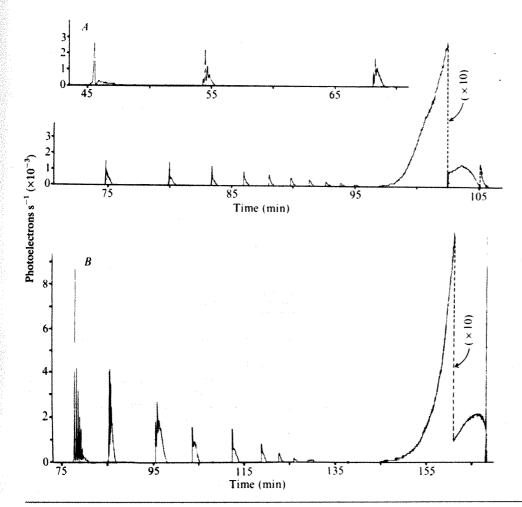


Fig. 2 Aequorin signals (photoelectrons s against time) from single zona-free oocytes in the presence of sperm (~10<sup>6</sup> per ml), recorded with digital photon-counting equipment. A, Sperm were added at 0 min. The first Ca<sup>2+</sup> transient is seen at 45 min, and a series of transients then follows before the dramatic Ca<sup>2+</sup> rise beginning at rise beginning at 97 min. Recorder sensitivity was reduced 10-fold at 102 min and returned to normal at 105 min. The trace ends at 106 min, the aequorin having been consumed completely. B, A similar experiment recorded at a slower chart speed. The trace has been re-drawn from 85 to 135 min to give a constant time scale. Note the oscillations in the first two transients. (The constant lower limit of the oscillations in the first transient is an artefact of the digital recording.) Throughout the series the transients decline in peak height and duration, and occur more frequently later in the series. The last few transients in each series are superimposed on a slow exponentially rising phase in the base level of [Ca<sup>2+</sup>]<sub>i</sub>, recorded at high gain from the analog output of the photon counter. This is not seen on the digital recording shown.

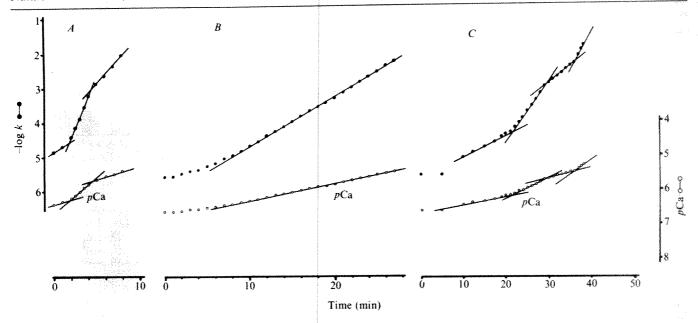


Fig. 3 A, B, fertilization data from Fig. 2 transformed into plots of −log k (●) and pCa (○) against time (see Fig. 1 legend for details), for the period of the large, 'activating' rise in [Ca<sup>2+</sup>]<sub>i</sub>. C, plots of -log k (•) and pCa (O) against time for the [Ca<sup>2+</sup>]<sub>i</sub>. l rise in an oocyte in Ca-free medium 16 containing 1 mM SrCl<sub>2</sub>. At the start of the plots shown, the oocyte had been in this medium for 45 min, with no change in the signal. Straight lines have been drawn through the points to show the piece-wise linearity of the plots.

Ca2+ release above that of Ca2+ sequestration would initiate an exponential rise in [Ca2+]i. This might be the first event in the process of activation, rather than a rise in [Ca<sup>2+</sup>], above a threshold. The probability of this event occurring may be increased by changes in [Ca<sup>2+</sup>], near the plasma membrane. This local Ca<sup>2+</sup> concentration may fall in Ca-free medium (because of reduced Ca<sup>2+</sup> influx) and rise with benzyl alcohol treatment and the Ca2+ transients seen in the fertilization experiments. Thus the initial effects of activating stimuli are various, but all serve to destabilize the system controlling  $R_{Ca}$ . Therefore the rises in [Ca<sup>2+</sup>], that result are similar.

Here we have given direct evidence that a large increase in [Ca<sup>2+</sup>] is an early event during activation of mouse oocytes. The time course of the [Ca<sup>2+</sup>], increase is an order of magnitude slower than the similar [Ca<sup>2+</sup>], increase seen in medaka<sup>1</sup> and sea urchin<sup>2</sup> oocytes. The data suggest that this [Ca<sup>2+</sup>], change depends on positive feedback which is itself controlled by an underlying system. An understanding of this system will be needed to explain fully the phenomenon of activation by various stimuli<sup>13</sup>. The technique used here to measure [Ca<sup>2+</sup>], in single mouse oocytes might provide a suitable model system for other cells where changes in [Ca2+], are important.

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#### **B-Endorphin and dynorphin control** serum luteinizing hormone level in immature female rats

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The suppressive effect of opioid administration on secretion of luteinizing hormone (LH) probably originates primarily in the hypothalamus<sup>1-5</sup>. Furthermore, the endogenous ligands of the opiate receptors (endorphins) seem to exert a tonic inhibition on the hypothalamic-pituitary-LH axis, as blockade of opiate receptors by the narcotic antagonist naloxone causes a pronounced increase in the release of LH3.4. However, it is unknown which of the particular endorphins present in the hypothalamus is responsible for this tonic inhibition. In addressing this issue we hypothesized that a similar effect to that obtained by blocking receptors with naloxone would be achieved by reducing the concentration of a specific endorphin at the receptors using antibodies against specific opioid peptides. We report here that both anti- $\beta$ -endorphin antibodies and anti-dynorphin<sub>1-13</sub> antibodies, when injected into the arcuate nucleus of the mediobasal hypothalamus (MBH) of immature female rats, caused an increase in serum LH. Anti-methionineenkephalin (Met-enkephalin) antibodies did not affect LH levels. We conclude that  $\beta$ -endorphin and dynorphin rather than Met-enkephalin have a role in regulation of LH secretion in immature female rats. These may represent the first data ascribing a specific physiological function to a particular endorphin.

Morphine, the enkephalins and  $\beta$ -endorphin exert their actions in the MBH by modulating the LH secreting activity of the pituitary<sup>3,5</sup>. The tonic inhibition effected by the endorphins

has been most convincingly documented in immature female rats: a dramatic (10-fold) increase in serum LH levels is seen within a few minutes of naloxone administration<sup>6,7</sup>. Assuming a competitive antagonism between naloxone and endorphins, the question remains as to which of the endogenous ligands of the opiate receptors actually controls the hypothalamus-pituitary-LH axis. This is of particular interest in view of the existence of a number of endorphins in the MBH<sup>8-11</sup>, which most probably correspond to multiple opiate receptors. In this respect, naloxone cannot provide more precise information as it acts almost equally well on each of the different opiate receptors. A promising system in which to investigate this issue seems, therefore, to be the extremely naloxone-sensitive hypothalamus-pituitary-LH axis of immature female rats. Microinjections into the MBH of specific anti-endorphin antibodies should neutralize the endorphins to give an elevation of serum LH, thus revealing the type of endorphin responsible for the tonic inhibition. This technique has been used successfully in the elucidation of different physiological mechanisms by administering antibodies either centrally 12-14 or peripherally 15

Anti-β-endorphin (titre 1:50,000; 50% binding of 15 fmol 125I tracer), anti-dynorphin<sub>1-13</sub> (1:20,000) and anti-Met-enkephalin (1:1,000) antisera were obtained and partially characterized as described elsewhere 10,19,20. As the titres of antidynorphin and anti-Met-enkephalin antisera were relatively low, we isolated the respective immunoglobulins by means of Protein A-Sepharose<sup>21</sup> and concentrated them to a final titre of 1:80,000. Anti- $\beta$ -endorphin antiserum (1:50,000) at a concentration of 1 μl was able to bind ~6 pmol <sup>125</sup>I-β-endorphin. The corresponding values were 8 pmol for the concentrated anti-dynorphin antibody (1:80,000) and 10 pmol for anti-Met-enkephalin antibody (1:80,000). The affinities of the antisera for their respective antigens were anti- $\beta$ -endorphin,  $7.0 \times 10^{11} \, \mathrm{l} \, \mathrm{mol}^{-1}$ ; anti-dynorphin  $1.1 \times 10^{10} \, \mathrm{l} \, \mathrm{mol}^{-1}$ ; and anti-Met-enkephalin,  $2.1 \times 10^{10} \, \mathrm{l} \, \mathrm{mol}^{-1}$ . CN-activated Sepharose 4B coupled to  $\beta$ -endorphin, dynorphin<sub>1-13</sub> and Met-enkephalin, respectively, was used to remove selectively the antibodies from the anti- $\beta$ -endorphin antiserum or the solutions containing the concentrated anti-dynorphin or anti-Met-enkephalin antibodies<sup>19</sup>. The remaining solutions completely lost their binding capacity and served as controls.

The powerful effect of naloxone (2.5 mg per kg s.c. (subcutaneous)) on serum LH levels in naive immature female rats<sup>6.7</sup> is confirmed here. LH levels increased to  $420\pm21$  ng ml<sup>-1</sup> (n=16) in 12-day rats (saline controls:  $43\pm19$ , n=24) 30 min after naloxone treatment and to  $381\pm16$  ng ml<sup>-1</sup> (n=11) in 14-day rats (controls:  $47\pm15$ , n=16). In 12-day female rats anaesthetized with hexobarbital (100 mg per kg s.c.) the antagonist increased the LH level to  $370\pm25$  ng ml<sup>-1</sup> (n=39) (saline controls:  $62\pm21$ , n=27).

In further experiments, unilateral microinjections (1 µl) of specific anti-endorphin antibodies were performed only on 12day female rats anaesthetized with hexobarbital (100 mg per kg s.c.). After 40 min the rats were decapitated, the blood collected and the brains removed. The serum LH concentrations determined were compared with the site of injection in the MBH, identified by histological examination. Figure 1 shows LH levels in rats after microinjection of different antibodies into distinct areas of the MBH. Figure 1a shows results for anti- $\beta$ -endorphin antiserum (titre 1:50,000); of nine areas injected, only injections into the arcuate nucleus were followed by an increase in LH (fourfold). A similar pattern was observed for antidynorphin<sub>1-13</sub> antibody (titre 1:80,000; Fig. 1b). Although the LH response after injection into the arcuate nucleus was less (twofold) than that observed with anti- $\beta$ -endorphin antiserum, the effect was highly significant (P < 0.01). Furthermore, LH levels doubled (P < 0.01) on injection of anti-dynorphin antibody into the nucleus dorsomedialis (pars ventralis); the control values for this area were the lowest of all obtained. Thus, although there was a statistically significant increase in LH in this case, the functional relevance of these data is questionable. In general, the control values were greater than those shown in

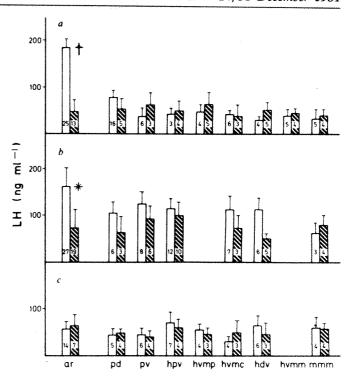


Fig. 1 Serum LH levels in 12-day-old female rats. The anaesthetized animals (hexobarbital, 100 mg per kg s.c.) were microinjected with: a, 1 µl anti- $\beta$ -endorphin antiserum (titre 1:50,000); b, anti-dynorphin<sub>1-13</sub> immunoglobulin-containing solution (1:80,000); or c, or anti-Met-enkephalin immunoglobulin-containing solution into different areas of the mediobasal hypothalamus (open columns). The hatched columns represent data from rats injected with 'carrier' solutions lacking the specific endorphin immunoglobulins. The animals were decapitated 40 min after the stereotaxically guided administration of the antibodies (5 µl Hamilton syringe, flat tip of the needle). Pilot studies on rats killed 20 min after antibody injection did not reveal changes in serum LH levels. The antibodies were injected within 3 min and the needle left in place for a further 3 min; experiments were done between 9.00 and 13.00 h. The trunk blood of the decapitated animals was collected, and the serum collected after 30 min at room temperature by centrifugation. The samples were stored at -20 °C and assayed in duplicate for LH as described in detail elsewhere26, using reagents provided by the Pituitary Agency of the National Institutes of Health. Variability within assays was 10% at an average level of 41 ng LHRP-1 (luteinizing hormone rat pituitary standard) per ml. The cannula position was verified by histological examination according to König and Klippel<sup>27</sup> Apparently, driving the needle through the median eminence caused extremely high serum LH levels (>800 ng ml<sup>-1</sup>). Anti-β-endorphin antibodies labelled with <sup>125</sup>I by the Chloramine-T method were used to study their diffusion within the hypothalamus. Punching the tissue around the site of injection revealed that the antibodies penetrated less than 1.8 mm from the site of injection within 40 min. The columns represent mean serum LH levels of different animals (number of animals shown within columns). Vertical bars indicate s.e. Significance of differences was calculated according to the two-tailed Student's t-test (\*P<0.01; †P<0.001). Data are given only for more than two injections into a certain area. The areas injected are indicated on the abscissa, abbreviated according to König and Klippel<sup>27</sup>: ar, nucleus arcuatus; pd, nucleus premamillaris dorsalis; pv, nucleus premamillaris ventralis; hpv, nucleus periventricularis (hypothalami); hvmp, nucleus ventromedialis (hypothalami), pars posterior; hvmc, nucleus ventromedialis (hypothalami), pars centralis; hdv, nucleus dorsomedialis (hypothalami), pars ventralis; hvmm, nucleus ventromedialis (hypothalami), pars medialis; mmm, nucleus mamillaris medialis, pars medialis.

Fig. 1a, c. We assume that this reflects the presence of impurities due to the processing of the anti-dynorphin antiserum. In contrast to the data obtained with anti- $\beta$ -endorphin and anti-dynorphin antisera, none of the areas injected with anti-Metenkephalin antibody (titre 1:80,000) responded with an increase in serum LH concentration (Fig. 1c). These results were obtained for high concentrations of antibodies (anti- $\beta$ -endorphin antiserum titre 1:50,000; anti-dynorphin<sub>1-13</sub> and anti-Met-enkephalin, each 1:80,000). Injections of  $1 \mu l$  anti- $\beta$ -endorphin antiserum diluted 10-fold (1:5,000) did not raise LH concentrations. Similarly, anti-dynorphin<sub>1-13</sub> antiserum (titre

1:20,000; not enriched) or anti-Met-enkephalin antiserum (1:1,000) failed to increase LH levels.

The observations reported here support the concept of a tonic inhibitory effect of endorphins on the hypothalamus-pituitary-LH axis. Two findings are of particular importance. First, LH levels are affected not only by anti- $\beta$ -endorphin antibodies but also, although to a lesser degree, by anti-dynorphin<sub>1-13</sub> antibodies. Second, the arcuate nucleus was the only sensitive region in a number of areas of the MBH tested. The fact that dopaminecontaining neurones and  $\beta$ -endorphin-containing neurones are closely associated in the arcuate nucleus<sup>22</sup>, together with our present findings, supports the notion of an interaction between endorphinergic and dopaminergic mechanisms in the modulation of the release of LH-releasing hormone from the median eminence<sup>1,2,5</sup>. The experiments implicating dynorphin in the control of serum LH via the arcuate nucleus are less compelling than the data suggesting a role for  $\beta$ -endorphin. However, in support of a regulatory role of dynorphin<sub>1-13</sub> are its high immunoreactive content in this area (1-5 pmol per mg protein)9-11 and its extremely high potency2

The finding that Met-enkephalin probably does not regulate serum LH in immature female rats is of interest in view of the conflicting data obtained from adult rats. Goldberg et al.2 reported that Met-enkephalin had no effect on serum LH concentrations, whereas others 1.2 have observed an inhibitory action of Met-enkephalin on the release of LH-releasing hormone in mediobasal hypothalamic slices. However, the Metenkephalin concentrations used in these studies were far greater than the K<sub>d</sub> value for Met-enkephalin (0.66 nM)<sup>25</sup>, therefore other receptors could also be activated by this opioid peptide. The general question remains whether mechanisms responsible for modulation of LH release in adult rats are similar or identical to those which operate in 12-day-old female

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#### Effects of nifedipine on electrical and mechanical responses of rat and guinea pig vas deferens

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The mechanical response of the vas deferens to single pulses or short trains of stimuli can be resolved into two distinct phases, an initial twitch response (I) attributed to the release of an unknown transmitter and a slower better maintained secondary contraction (II)1,2, attributed to noradrenaline release. Recently, it has been shown that the calcium antagonist nifedipine3 eliminates the initial twitch response leaving the secondary component intact<sup>4,5</sup> and we have now examined the electrical responses underlying these mechanical events. We show here that nifedipine abolishes the smooth muscle action potential and the initial twitch response (I) without reducing the excitatory junction potential (e.j.p.), suggesting a fundamentally different basis of the two responses and illustrating the pharmacological value of nifedipine.

In rat and guinea pig, the contractile response of the vas deferens to a single field stimulus to the sympathetic innervation consisted of several components. In the rat this response could be separated into two components; a rapid, early (200-300 ms) response  $(I_s)$  and a slower (600-700 ms) response  $(II_s)$ . The  $\alpha_1$ -adrenoreceptor antagonists, prazosin  $(10^{-7} \,\mathrm{M})$  or corynanthine  $(3 \times 10^{-6} \text{ M})$ , blocked  $II_s$  but left  $I_s$  intact. In contrast, nifedipine  $(10^{-5} \text{ M})$  together with  $\alpha_1$ -adrenoreceptor antagonists virtually eliminated the whole response (Fig. 1).

In the guinea pig, where separation of the mechanical response to single stimuli by time course into two distinct components is less clear than in the rat2.6, corynanthine had little effect but nifedipine reduced the response by 40-70% and, by producing a relatively greater inhibition of the early part of the contraction, altered the time course. Thus, in each species the contractile response to a single stimulus contained a nifedipinesensitive but  $\alpha_1$ -antagonist-resistant component  $(I_s)$  and an  $\alpha$ -noradrenergic component  $(H_s)$ .

In each species, a train of 25 pulses at 6 Hz produced a biphasic concentration, a rapid, early (1-2s) component  $(I_t)$ followed by a second component  $(II_t)$ , which lasted until stimulation ceased, I, and II, being relatively dominant in the prostatic and epididymal portions, respectively<sup>6,7</sup>. In each species, corynanthine (3×10<sup>-6</sup> M) produced inhibition which was greater in II, but which, again, did not exceed 50%. In contrast, nifedipine, although reducing each component, produced a particularly marked reduction of up to 90% in  $I_{\rm b}$ . The effects of nifedipine and corynanthine were additive but did not result in complete abolition of the response. Thus, in each species, each phase of the response contained a nifedipine-sensitive component which was a dominant factor in  $I_t$ . In contrast the component attributed to  $\alpha$ -noradrenergic activation contributed mainly to  $H_t$ , particularly in the guinea pig where the noradrenergic component to single pulse is less obvious. The nifedipine-sensitive component corresponded in drug sensitivity, time course and species specificity to the nerve-induced 'non-noradrenergic' contraction 1,2,6,7

In separate experiments on the guinea pig vas deferens, e.j.ps and action potentials were recorded using intracellular microelectrodes (20-40 M $\Omega$  filled with 3M KCl) following pre- and

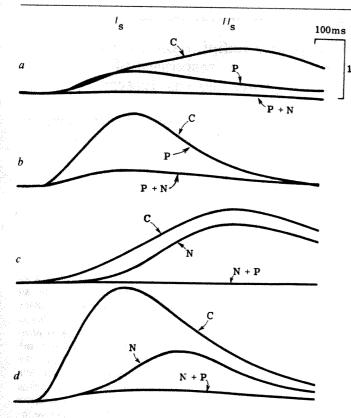


Fig. 1 Effects of drugs on the time courses of the isometric contractions of isolated portions of rat vas deferens to single supramaximal field stimuli (0.5 ms). The trace was triggered by the stimulus. a, The epididymal and b, the prostatic portions of a vas deferens. Control (C) was followed by responses in the presence of prazosin  $(10^{-7} \text{ M})$  (P) and then with the further addition of nifedipine  $(10^{-5} \text{ M})$  (P+N). In this particular experiment, prazosin had no effect at all in b so C and P coincide. c, d, The contralateral vas—as for a and b except that nifedipine (N) was administered first and was followed by the addition of prazosin (N+P).  $I_s$  and  $I_s$  indicate the times of the peaks of the two phases of contraction.

postganglionic hypogastric nerve stimulation with trains of stimuli at 5-10 Hz, pulse width 0.2 ms and supramaximal voltage<sup>8</sup>.

In all preparations, trains of stimuli at 5 Hz evoked e.j.ps which by facilitation and summation reached threshold for the initiation of a muscle action potential and the vas deferens contracted violently, normally dislodging the microelectrode. The muscle action potential had an overshoot of >15 mV in all control cells impaled. At 10-15 min after nifedipine (10<sup>-5</sup> M to  $3 \times 10^{-5}$  M), the e.j.p. was not reduced, and if anything was enhanced. Trains of stimuli now produced a depolarization which greatly exceeded the control threshold, but it was not possible to induce muscle action potentials (Fig. 2) even with trains of stimuli at 10 Hz. Nifedipine reduced the rate of rise and fall of the spike before complete block occurred (Fig. 3). During long trains of stimuli at 5 and 10 Hz, the microelectrode was dislodged from the cell presumably due to contraction of the vas deferens, even though no action potentials were recorded. After wash-out (15 min) the muscle action potential began to return (Fig. 2) although the rate of rise was still greatly reduced compared with the control.

The  $\alpha$ -adrenoreceptor antagonist prazosin ( $10^{-6}$  M) did not reduce the muscle action potential but increased e.j.p. amplitude. Prazosin did not prevent the effects of nifedipine ( $3 \times 10^{-5}$  M), suggesting that nifedipine is not acting via an  $\alpha$ -adrenoreceptor.

Previously, it has not been possible to block selectively the initial twitch and leave the secondary response (II) intact. We have now shown that nifedipine blocks the initial twitch (I) and prevents the initiation of the smooth muscle action potential.

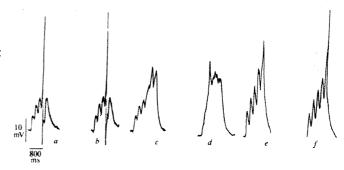


Fig. 2 Effects of nifedipine  $(10^{-5} \text{ M})$  on excitatory junction potentials and action potentials evoked in the guinea pig vas deferens by hypogastric nerve stimulation (pulse width 0.2 ms, supramaximal voltage). Stimulus artefacts have been removed from the trace. a, b, Controls, resting membrane potential (RP) – 57 mV and –56 mV respectively. Response evoked by trains of five pulses at 5 Hz. c, 15 min after nifedipine; RP = -62 mV, six pulses at 5 Hz. d, Same cell as d, 10 pulses at 10 Hz. d, Wash 10 min; RP = -63 mV, five pulses at 5 Hz. d, Same cell as d, 15 min wash; five pulses at 5 Hz. Records d/d, d/d and d/d are taken from three neighbouring cells in the same preparation.

Nifedipine is known to be a calcium antagonist without local anaesthetic activity and it is also known that calcium is the ion carrying most of the inward current during the rising phase of the action potential  $^{10-12}$ . The initial twitch response (I) therefore corresponds to the contraction associated with the smooth muscle action potential. Since the e.j.p. initiates the smooth muscle action potential and associated contraction, and is resistant to concentrations of  $\alpha$ -adrenoreceptor antagonists which block the noradrenergic contraction, it is probable that the  $\alpha$ -adrenoreceptor mediated contractile response of the vas deferens does not require an action potential.

These results show there to be a fundamental difference between the two types of response to nerve stimulation in the vas deferens. If noradrenaline is the sole transmitter then it

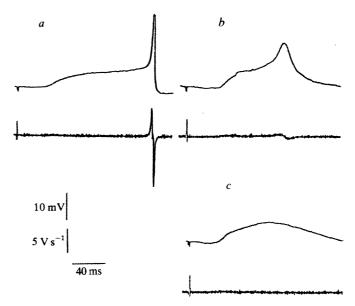


Fig. 3 The effects of nifedipine  $(10^{-5} \text{ M})$  on action potentials in the guinea pig vas deferens. Action potentials were evoked by stimulation of the hypogastric nerve with trains of five stimuli at 5 Hz. The records show the membrane response initiated by the fourth stimulus in a train. The upper record is the d.c. response calibration bar 10 mV; lower record, the differential of the d.c. signal, calibration bar  $5 \text{ Vs}^{-1}$ . a, Control, resting membrane potential (RP) -62 mV; b, 10 min after nifedipine, RP = -64 mV. Records a, b and c are taken from three neighbouring cells in the same preparation.

produces its contractile effects through two processes; the first involves e.j.ps which summate to initiate propagated action potentials and does not involve  $\alpha$ -adrenoreceptors; the second does not involve e.j.ps or propagated action potentials but is mediated by  $\alpha_1$ -adrenoreceptors. The only experimental obstacle which remains to acceptance of noradrenaline as sole transmitter is the survival of the first process after reserpine or chemical sympathectomy<sup>13</sup>. The results provide further proof that the different types of response in the two ends of the vas result from differences in the dominant transmission process.

These results in vas deferens show some similarities with recent data derived from arteriolar smooth muscle 14,15. First, in

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each case the e.j.p. was found to be resistant to  $\alpha_1$ -adrenoreceptor antagonists. Second, in the responses which are mediated by  $\alpha_1$ -adrenoreceptors in these tissues, there is a similarity between the contractile response of the arterioles to iontophoretically applied noradrenaline, which occurred without any detectable change in membrane potential, and the 'adrenergic' response to nerve stimulation in the vas, which occurred independently of the action potential. This suggests that in smooth muscle there is a type of  $\alpha_1$ -adrenoreceptor which may directly activate the contractile process without utilising the nifedipine-sensitive influx of calcium associated with the rising phase of the action potential.

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#### Vertical organization of neurones accumulating 3H-GABA in visual cortex of rhesus monkey

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Electrophysiological and pharmacological studies 1-6 indicate that the specific responses of most visual cortical neurones depend on intracortical y-aminobutyric acid (GABA)-mediated inhibitory processes. GABAergic interneurones have been visualized in all layers of the mammalian cerebral cortex by immunocytochemical methods 7.8 and by high-affinity uptake of exogenous 3H-GABA9-11. It is recognized that GABA is synthesized and specifically accumulated by aspinous and sparsely spinous stellate cells, but there is no evidence available to indicate whether the laminar distribution of these cells and their axonal projections are related to the known role of GABAergic inhibitory processes in the generation of responses in visual cortical cells. It would therefore be of value to delineate the intracortical projection of the axons of different types of GABA-releasing neurones in regions of cortex where the receptive field properties of the neurones, and their modification by GABA antagonists, are well known. The selective highaffinity uptake of labelled GABA has been useful in delineating GABAergic systems12: recently, it has been shown that exogenous 3H-GABA is specifically taken up and transported retrogradely by axons of neurones thought to be GABAergic 13,14. Using microinjections of 3H-GABA into different layers of the monkey visual cortex, we have examined the pattern of labelled neurones. We report here a bimodal distribution of GABA-accumulating neurones after injection into layers V and VI, with one group of neurones around the injection site and the other directly above, in layers II and III. We provide evidence that the latter neurones are non-pyramidal cells, probably labelled by retrograde axonal transport from the deep lavers.

Two adolescent male macaque monkeys were used. One had taken part in behavioural tests of memory and had received surgical section of the fornix 6 months before the present study. The other had a high antibody titre to Herpes simiae and could therefore not be used in long-term experiments. There was no reason to suppose that the visual cortex of either monkey was abnormal. The animal was sedated with an intramuscular injection of ketamine hydrochloride (10 mg per kg; Ketalar, Parke-Davis) and then deeply anaesthetized with an intravenous injection of sodium pentobarbitone (Sagatal, May and Baker). After exposing the lateral surface of one occipital lobe, the cortex was injected with <sup>3</sup>H-GABA (0.33 mM, 60 Ci mmol<sup>-1</sup>; Radiochemical Centre) dissolved in Krebs bicarbonate<sup>15</sup>. All the injections were delivered using glass micropipettes (30-50 µm tip diameter) penetrating at a very oblique angle to the surface of the cortex. The pipette was advanced 7-9 mm from the pia and 0.1 µl (2 µCi) 3H-GABA injected by pressure16 at each of four to six sites at 1-2 mm intervals along the injection track as the capillary was gradually withdrawn. In the first monkey the GABA injection track (no. 1) in area 17 passed obliquely through layers V and VI and ended in the white matter. In the other monkey one injection track (no. 2) in area 17 advanced obliquely from layer I to layer IVA, and another injection track (no. 3) in area 18 (posterior lip of the lunate sulcus) included all layers and the white matter as the capillary proceeded gradually deeper. After a post-injection survival time (no. 1, 40 min; no. 2, 55 min; no. 3, 35 min) the animals were perfused with fixative and slices of the injected cortex processed for Golgi staining and gold toning, as described previously16. Semi-thin (1 µm) sections perpendicular to the injection track were cut from the Golgi sections and processed for autoradiography<sup>11</sup>. Golgi staining was used to reveal the processes of some of the GABA-accumulating cells, but because of the unreliability of the staining, no unequivocal identification of the cell type of neurones labelled in the upper layers has yet been obtained.

Neurones in each of layers I-VI in both areas 17 and 18 became labelled when the layer was injected with <sup>3</sup>H-GABA. The number of labelled cells gradually decreased in all directions from the injection track, but labelled cells could always be found above the capillary track up to and including layer II. A distinct, bimodal pattern of labelled neurones and fibres was observed when the injection track passed through lower layer V, layer VI and the superficial white matter (injections 1 and 3; Fig. 1a). Large labelled neurones were seen around the injection site with scattered neurones as far as 1-1.5 mm from the injection site in layers V and VI. Occasionally, labelled neurones were found in layer IV and lower layer III, always above the injection track. In addition, another group of strongly labelled small, mainly fusiform cells was observed in layers II and upper III (Fig. 2a, b). The quantitative distribution of the neurones labelled by one oblique penetration in area 17 is shown in Fig. 1b.

It is unlikely that the neurones in the upper group were labelled by GABA taken up from the extracellular space in the upper layers, because there were very few labelled neurones in lower layer III and layer IV, although these layers contain a

large number of GABA-accumulating neurones when injected directly. A more likely explanation is that the neurones in layers II and upper III were labelled by retrograde axonal transport of <sup>3</sup>H-GABA from the injection site in the deep layers. This is supported by the observation that strongly labelled fibre bundles pass through layers IV and lower III (Fig. 2c).

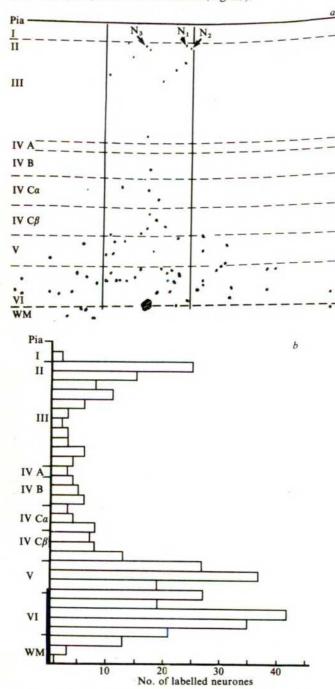


Fig. 1 a, Location of labelled neuronal perikarya (dots) in a single section of monkey striate cortex after 3H-GABA injection at the border of layer VI and the white matter. The drawing is of a section perpendicular to the pial surface; the injection was made obliquely at an angle of almost 90° to the plane of the section. Besides the large labelled neurones around the injection track (shaded area) and a few neurones in layer IV, there is a group of small labelled neurones in layer II and upper layer III. N1-N3 are shown on light micrographs in Fig. 2. Note the vertical alignment or labelled neurones above the injection track. The vertical lines enclose a 500 µm column in which cells were counted for b. b. Distribution of 393 3H-GABA-labelled cells in all layers (I-VI) of the striate cortex, counted in 13 semi-thin sections cut perpendicular to the injection track at different levels of injection no. 1, Thick vertical line represents the levels where 3H-GABA was injected. WM, white matter.

What type of neurones in the upper layers accumulate GABA from the deeper layers? There is a very strong projection from layer II to layer V in area 17 of the monkey, mediated by small pyramidal cells<sup>17</sup>. Thus the terminals of these neurones could take up the injected GABA. As pyramidal and stellate neurones can be differentiated on the basis of fine structural criteria, we studied the characteristics of six labelled neurones from the upper layers. After localizing the labelled neurones in semi-thin sections, a series of 84 and 132 ultrathin sections, respectively, were cut from two blocks containing the remainder of the perikarya of the labelled neurones. Using electron microscopy we found that labelled neurones had an indented eccentric nucleus with dense clumps of chromatin. There were many free polyribosomes in the cytoplasm, making the cell conspicuously electrodense. The thin dendrites emerging from the perikarya contained densely packed microtubules (Fig. 2d) and some of the dendrites were beaded, receiving predominantly asymmetric

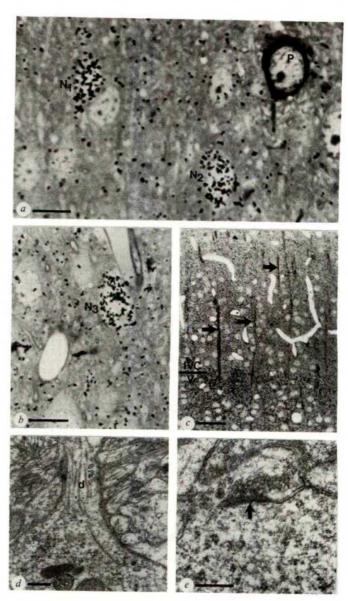


Fig. 2 Light (a-c) and electron microscopic (d,e) details from the area shown in Fig. 1a. a, b, Semi-thin sections of  ${}^{3}\text{H-GABA-labelled}$  neurones  $(N_{1}-N_{3})$  in layer II, which were also serially sectioned for electron microscopy. One Golgi-stained pyramidal cell (P) is also present. c, Vertical labelled fibre bundles (arrows) passing through layer IVC. d, Origin of a small dendrite (d) from the neurone  $N_{3}$  directed towards the pia. e, Asymmetric synapse on the perikaryon of the labelled neurone  $N_{2}$ . a-c, Ilford K5 emulsion; 30 days exposure. Scale bars: a, b,  $10~\mu\text{m}$ ; c,  $50~\mu\text{m}$ ; d, e,  $0.2~\mu\text{m}$ .

synapses. The perikarya received few, mainly symmetrical synapses from boutons containing pleomorphic vesicles, but occasional asymmetric synapses established by boutons containing round synaptic vesicles were also observed (Fig. 2e). All these features are characteristic of small stellate cells in the monkey primary sensory cortex<sup>18,19</sup>. For comparison we also studied two Golgi-stained, gold-toned small pyramidal cells in layer II. These neurones differed from labelled cells in that the nucleus was large, round and relatively transparent and there were few free ribosomes. Thick dendrites tapered gradually from the perikaryon and an apical dendrite was observed. The perikarya and proximal dendrites received only symmetrical synapses, confirming previous findings<sup>20,21</sup>

These data taken together provide evidence for a GABAaccumulating stellate neurone system with perikarya situated in layer II and upper layer III, and with axons descending vertically in bundles into layer V. One stellate neurone, the doublebouquet cell with tight vertical axon bundles described in previous studies<sup>22-25</sup>, corresponds very well to the above characteristics but more detailed information is required before we can conclude definitely that GABA-accumulating neurones of the upper layers are, or include, double-bouquet cells. It was shown recently25 that this neurone makes symmetrical synaptic contacts, similar to those which contain glutamic acid decarboxylase (GAD) in the monkey<sup>26</sup>, with dendritic shafts and spines. Although the axons of double-bouquet cells do not enter layer VI the fact that an injection here produces labelled neurones in layers II and III is easily explained by diffusion of the GABA across the V-VI boundary.

The presence of a GABAergic vertical neurone system passing throughout layers II to V of the visual cortex and capable of influencing neurones in layer VI with dendrites in layer V is significant in view of the functional, columnar organization of cortical neurones<sup>27</sup>. The distribution of GAD immunoreactivity in the plane of the cortical surface was recently shown to follow a pattern resembling ocular dominance columns7. On the other hand, it has been shown in area 17 that cortical neurones in a radial column are maximally excited by a bar of light or grating of a particular orientation, and that this orientation specificity is temporarily abolished by the GABA antagonist bicuculline<sup>2</sup> The columnar system of GABA-accumulating axons described here may have an important role in this specificity, as the axon bundles are highly restricted in lateral extent (20-50 µm in diameter) and could have a powerful effect locally.

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### Agonist and antagonist benzodiazepine receptor interaction in vitro

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A representative of a novel series of imidazodiazepines, Ro 15-1788, selectively antagonizes all major central actions of benzodiazepines by competitive, high-affinity interactions with benzodiazepine receptors (BR) in the central nervous system (CNS)1-3. Doses of Ro 15-1788 sufficient to antagonize benzodiazepine actions have been shown per se to lack pharmacological action in animals and man1-4. Thus Ro 15-1788 provides a highly selective tool for experimental investigations of BR-mediated events and is of therapeutic value in all cases where a rapid termination of benzodiazepine actions is indicated. Using <sup>3</sup>H-labelled Ro 15-1788 as radioligand in equilibrium binding studies in vitro, we show here that 3H-Ro 15-1788 interacts with the same number of BR sites as the agonist 3H-clonazepam in various brain regions. However, their mode of receptor interaction is different. In conditions which alter receptor affinity for 3H-clonazepam binding, such as addition of  $\gamma$ -aminobutyric acid (GABA) or certain ions, no change is seen in 3H-Ro 15-1788 binding. This effect can be used to distinguish between benzodiazepine receptor agonists and antagonists in vitro. Furthermore, the thermodynamics of agonist and antagonist receptor interaction are different, but only at temperatures above 21 °C.

Several lines of evidence indicate that <sup>3</sup>H-Ro 15-1788 interacts with the same central type of BR sites as benzodiazepine

(1) <sup>3</sup>H-Ro 15-1788 was bound to the same maximal number of specific binding sites  $(B_{\text{max}})$  as  $^{3}\text{H-clonazepam}$  in frozen and extensively washed synaptic membrane fractions<sup>5</sup> prepared from various brain regions. 3H-clonazepam was used as agonist because, in contrast to 3H-flunitrazepam (3H-FNZP) and 3Hdiazepam, it binds selectively to the central type of BR without interacting with the peripheral type of benzodiazepine binding sites present in brain tissue<sup>6</sup>. Scatchard analysis of <sup>3</sup>H-Ro 15-1788 and <sup>3</sup>H-clonazepam specific binding revealed only one population of binding sites in each brain area tested, with no major regional difference in the apparent affinity constant  $(K_D)$ for either radioligand (cerebral cortex: 3H-Ro 15-1788 (3Hclonazepam) specific binding,  $B_{\text{max}} = 1.7(1.6)$  pmol per mg protein,  $K_D = 0.9(0.9)$  nmol 1<sup>-1</sup>; cerebellum:  $B_{\text{max}} 0.7(0.7)$ ,  $K_D =$ 0.8(0.7); hippocampus: 1.7(1.6), 0.9(0.8); hypothalamus: 1.2(1.2), 0.9(1.0); striatum: 0.9(0.8), 1.1(1.0); midbrain: 0.9(0.7), 1.1(0.9); medulla pons: 0.6(0.5), 0.8(0.8). Values of s.e.m. from at least three determinations of  $K_D$  or  $B_{max}$  were in the range  $\pm 0.08$ –0.1. In  $^3$ H-Ro 15-1788 binding the antagonist Ro 14-7437 (1μmol I<sup>-1</sup>), which is the desfluoro analogue of Ro 15-1788, was used as displacer<sup>2</sup>. <sup>3</sup>H-clonazepam was displaced by 10 μmol l<sup>-1</sup> diazepam).

(2) Autoradiographically, the density and distribution of <sup>3</sup>H-Ro 15-1788 binding sites in sections of various CNS regions were very similar to those seen with <sup>3</sup>H-clonazepam.

(3) A variety of receptor agonists and antagonists showed very similar potency in inhibiting either 3H-clonazepam or 3H-Ro 15-1788 binding (Table 1).

(4) The inhibition of <sup>3</sup>H-Ro 15-1788 binding by diazepam or flunitrazepam was competitive1.

(5) In heat inactivation experiments, the amount of specifically bound <sup>3</sup>H-Ro 15-1788 was reduced at the same rate and to the same extent as that of 3H-clonazepam (60 °C, 5-60 min). In the presence of 10  $\mu$ mol l<sup>-1</sup> of GABA, diazepam or

Table 1 Inhibition of <sup>3</sup>H-clonazepam and <sup>3</sup>H-Ro 15-1788 binding by various BR ligands

	IC <sub>50</sub> (n	imol l <sup>-1</sup> )
	<sup>3</sup> H-clonazepam	<sup>3</sup> H-Ro 15-1788
Ligand	binding	binding
Agonists		
Clonazepam	1.2	1.4
Flunitrazepam	5.1	4.8
Diazepam	13.5	19.5
(+) Ro 11-6896	15.5	14.5
Zopiclone	46	50
C1 218 872	300	340
Chlordiazepoxide	1,300	1.100
Medazepam	1,900	1,900
(-) Ro 11-6893	>10,000	>10,000
Antagonists		
Selective		
Ro 15-1788	2.0	1.3
Ro 14-7437	4.5	3.0
Non-selective	***	5.0
CGS 8216	0.3	0.2
Ethyl-β-CC	1.6	2.0
Methyl-β-CC	2.3	1.9

Inhibition of  $^3$ H-clonazepam binding (specific activity 16.1 Ci mmol $^{-1}$ , 0.5 nmol  $l^{-1}$ ; assay: H.M., in preparation) and of  $^3$ H-Ro 15-1788 binding (specific activity 26.8 Ci mmol $^{-1}$ , 0.5 nmol  $l^{-1}$ ; ref. 2) by various BR ligands at 0 °C using freeze-thawed and extensively washed synaptic membranes $^5$  of rat cerebral cortex. The concentrations giving half-maximal inhibition (IC<sub>50</sub>) are mean values of three determinations with s.e.m. values between  $\pm 5$  and 20%. (For the structure of enantiomers Ro 11-6896 and Ro 11-6893, of CGS 8216, CL 218 872 and zopiclone, see refs 27, 22, 16 and 15, respectively.)  $\beta$ -CC,  $\beta$ -carboline-3-carboxylate.

Ro 15-1788, the binding sites for the two radioligands were similarly protected from heat inactivation (60 °C, 30 min).

(6) Ro 15-1788 did not interact with the peripheral type of benzodiazepine binding site in the brain as it was unable to displace  ${}^{3}\text{H-Ro}$  5-4864 when tested in cerebral cortex (IC<sub>50</sub> > 10  $\mu$ mol l<sup>-1</sup>)<sup>1</sup>.

Thus, Ro 15-1788 seems to interact with the same number of sites of the central BR type as benzodiazepine agonists. The mode of interaction of Ro 15-1788 with BR is, however, different from that of receptor agonists. BR affinity for <sup>3</sup>H-diazepam or <sup>3</sup>H-FNZP binding is increased in the presence of various chemicals, including  $100 \,\mu\text{mol}\,l^{-1}$  GABA<sup>8.9,28-30</sup>,  $500 \,\mu\text{mol}\,l^{-1}$  pentobarbitone <sup>10,11</sup>,  $10 \,\mu\text{mol}\,l^{-1}$  SQ 20 009 (ref. 12),  $2 \,\text{mmol}\,l^{-1}\,\text{NiCl}_2$  (ref. 13),  $20 \,\text{mmol}\,l^{-1}\,\text{NH}_4\text{I}$ ,  $200 \,\text{mmol}\,l^{-1}\,\text{NH}_4\text{Cl}$  or  $100 \,\text{mmol}\,l^{-1}\,\text{NH}_4\text{Br}$  (ref. 14), while the BR affinity is decreased in the presence of 50 mmol  $l^{-1}\,$  (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> or  $200 \,\text{mmol}\,l^{-1}\,$  ammonium acetate <sup>14</sup>. However, in parallel assays, neither the  $K_D$  nor the  $B_{\text{max}}$  value for <sup>3</sup>H-Ro 15-1788 binding was affected by these agents. This lack of susceptibility of <sup>3</sup>H-Ro 15-1788 binding to conformational changes of BR may reflect the inability of Ro 15-1788 to induce a conformational change of the receptor and, thereby, to trigger a biological response

of the receptor and, thereby, to trigger a biological response. The different responses of <sup>3</sup>H-agonist and <sup>3</sup>H-Ro 15-1788 binding to alterations in BR affinity can be used to distinguish in vitro between agonists and antagonists; the inhibitory potency of a ligand is determined in <sup>3</sup>H-Ro 15-1788 binding in the presence and absence of a compound such as GABA which is known to alter the affinity for agonists. At 35 °C the addition of 100 μM GABA increased two- to threefold the inhibitory potency (IC<sub>50</sub>) of various BR agonists such as flunitrazepam, diazepam, oxazepam, zopiclone <sup>15</sup> and Cl 218 872 (ref. 16) in <sup>3</sup>H-Ro 15-1788 binding, while the inhibitory potency of various antagonists remained unaltered, as shown for the selective antagonists Ro 15-1788 and Ro 14-7437 (Fig. 1) and for CGS 8216 and ethyl-β-carboline-3-carboxylate, which block non-selectively at least some benzodiazepine actions <sup>17-22</sup>. The inhibitory potency of methyl-β-carboline-3-carboxylate, an

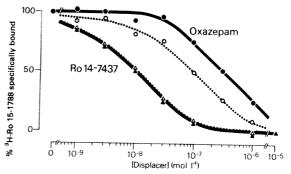


Fig. 1 Inhibition of <sup>3</sup>H-Ro 15-1788 binding in the presence (open symbols) and absence (solid symbols) of 100 µmol 1 GABA, by the agonist oxazepam (circles) and the antagonist Ro 14-7437 (triangles) at 35 °C using freeze-thawed and extensively washed synaptic membranes of rat cerebral cortex. IC<sub>50</sub> values ± s.e.m. of at least three determinations in the absence (presence) of GABA were: flunitrazepam 27.6±6 (8.2±0.3 P<0.02); diazepam 94± 11 (32.5 ± 5.0; P < 0.01); zopiclone  $160 \pm 10(97 \pm 10; P < 0.02)$ ; oxazepam  $315 \pm 10$  ( $116 \pm 14$ ; P < 0.01); CL 218  $872:560 \pm 30$  $(350\pm20; P<0.01)$ . Ro 15-1788:  $7.4\pm0.8$   $(6.9\pm0.5)$ ; Ro 14-7437:  $13.2 \pm 1.9$  (14.6  $\pm 0.3$ ); CGS 8216:  $0.44 \pm 0.1$  (0.61  $\pm 0.1$ ); propyl- $\beta$ -CC: 11.9  $\pm$  0.3 (11.6  $\pm$  0.4); ethyl- $\beta$ -CC 5.5  $\pm$  0.8 (7.9  $\pm$ 0.6), all with no significant difference by Student's t-test. Methyl- $\beta$ -CC,  $8.8 \pm 0.8$  (14.8  $\pm$  1.0; P < 0.02). Similar results were obtained at 0 °C, except that the IC50 values of zopiclone and methyl-β-CC remained unchanged in the presence of GABA.  $\beta$ -CC,  $\beta$ -carboline-3-carboxylate.

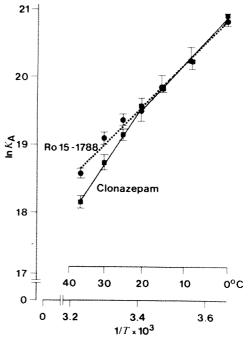


Fig. 2 Van't Hoff plot of the dependence of  $K_{\rm A}$  of  $^3{\rm H-Ro}$  15-1788 and  $^3{\rm H-clonazepam}$  binding on temperature (T), using frozen and extensively washed synaptic membranes of rat cerebral cortex. Each point represents the mean  $(\pm\,{\rm s.e.m.})$  of three determinations. The apparent dissociation constant  $K_{\rm D}$   $(1/K_{\rm A})$  for  $^3{\rm H-clonazepam}$   $(^3{\rm H-Ro}$  15-1788) at 0 °C were:  $0.86\pm0.03$   $(0.87\pm0.07)$  nmol  $1^{-1}$ ; at 7.5 °C:  $1.68\pm0.2$   $(1.72\pm0.33)$ ; 15 °C:  $2.46\pm0.12$   $(2.48\pm0.41)$ ; 20 °C:  $3.25\pm0.38$   $(3.55\pm0.46)$ ; 25 °C:  $4.92\pm0.39$   $(3.98\pm0.35)$ ; 30 °C:  $7.46\pm0.78$   $(5.16\pm0.42)$ ; 37 °C:  $13.2\pm1.3$   $(8.64\pm0.57)$ . The  $B_{\rm max}$  values did not change with temperature. The thermodynamic parameters for  $^3{\rm H-clonazepam}$   $(^3{\rm H-Ro}$  15-1788) binding at 37 °C were:  $\triangle G^0 = -11.20$  (-11.46) kcal mol $^{-1}$ ;  $\triangle H^0 = -14.98$  (-9.87) kcal mol $^{-1}$ ;  $\triangle S^0 = -12.20$  (-5.12) EU. The parameters at 0 °C were:  $\triangle G^0 = -11.34$  (-11.34) kcal mol $^{-1}$ ;  $\triangle H^0 = -10.34$  (-9.87) kcal mol $^{-1}$ ;  $\triangle S^0 = +3.65$  (+5.3) EU (entropy units).

antagonist having convulsant activity23, was even decreased in the presence of GABA (see Fig. 1 legend). This test may be relevant not only for differentiating agonists from antagonists, but also for evaluating mixed agonist-antagonists.

The thermodynamics of the receptor binding reaction for Ro 15-1788 differ from those of clonazepam as shown by the change in standard Gibbs free energy ( $\Delta G^{\circ}$ ), in entropy ( $\Delta S^{\circ}$ ) and enthalpy  $(\Delta H^{\circ})$  determined as described elsewhere<sup>2</sup> van't Hoff plot, from which  $\Delta H^{\circ}$  is calculated, showed an inclination point at 21 °C for 3H-clonazepam binding (as for other agonists<sup>25,26</sup>) but was linear for <sup>3</sup>H-Ro 15-1788 (Fig. 2). Above 21 °C, <sup>3</sup>H-clonazepam binding was associated with a large decrease in enthalpy which compensated for an unfavourable decrease in entropy. 3H-Ro 15-1788 binding, however, was enthalpy- as well as entropy-driven (see Fig. 2 legend). These

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findings agree with a two-step model of receptor binding, as proposed for other receptors<sup>24</sup>:  $L+R \stackrel{(1)}{\longrightarrow} LR \stackrel{(2)}{\longrightarrow} LR'$  where L is the ligand and R the receptor. Agomsts and antagonists may bind in reaction (1) with similar thermodynamic components. Due to their structural properties, agonists, but not antagonists, can participate in reaction (2) by inducing a conformational change of the receptor to R'. This conversion is thought to be associated with negative enthalpy and entropy components. Below the temperature of 21 °C, at which phase transitions of membrane lipids are known to occur, the thermodynamic parameters of <sup>3</sup>H-clonazepam binding were almost identical to those of <sup>3</sup>H-Ro 15-1788 binding (see Fig. 2 legend). Possibly the agonist-dependent change in BR conformation cannot be induced at temperatures <21 °C.

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#### Immunohistochemical mapping of vitamin D-dependent calcium-binding protein in brain

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The steroid hormone, 1, 25-dihydroxycholecalciferol (1, 25-(OH)2D3) causes the de novo synthesis of a calcium-binding protein (D-CaBP)1,2. This protein is present in highest concentrations in intestine, kidney and shell gland, across which calcium is transported in relatively large amounts, but it is also found in smaller amounts in several other tissues, including brain<sup>3-7</sup>. The area of brain with the highest D-CaBP concentration is the cerebellum, where the protein is found only in the Purkinje cells<sup>7,8</sup>. We have now mapped D-CaBP immunohistochemically throughout the brain of chicks and rats and present here a list of all positive nuclei. Because certain of these neurones also contain 1, 25-(OH)<sub>2</sub>D<sub>3</sub> (ref. 9), we suggest that they are target cells for this hormone, thus broadening the functional significance of vitamin D to include the brain and implicating vitamin D in a more widespread action than simply a role in the calcium translocation mechanism of epithelial cells.

Intestinal D-CaBP from both birds and animals has been well characterized with respect to its molecular weight, calciumbinding constants and electrophoretic mobility 1,10,11. D-CaBP from chick brain resembles that of chick intestine immunologically, in molecular weight and in electrophoretic mobility and is "indeed the same protein" 12. The antisera against chick intestinal D-CaBP give precipitin lines with rat and bovine brain D-CaBP<sup>13</sup> and consequently rat brain can also be stained for this protein. We have taken sections from every area of rat and chick brains and assessed them for D-CaBP-positive nuclei by an immunoperoxidase technique8. The protein was found in various cells throughout the brain of these two species but only in neuronal elements and never in glial, endothelial or ependymal cells. Within any brain region it was always localized in certain specific neuronal types (Table 1 and Fig. 1a-c)

There seems to be no common factor between the D-CaBPpositive neurones listed in Table 1 that could suggest a function for this protein. However, in three locations, on the basis of electrophysiological data available, there seems to be a special need for calcium regulation. The Purkinje cells of the cerebellum<sup>14,15</sup>, the neurones of the inferior olive<sup>16</sup> and CA1 pyramidal cells of the guinea pig hippocampus<sup>17</sup> produce voltage-dependent calcium spikes within their dendritic trees. These then induce the usual sodium-dependent action potentials from the soma-initial segment of each of these neurones. All the neurones contain D-CaBP, both in their soma and dendrites (Fig. 1a-c). The pyramidal neurones of CA3 also produce voltage-dependent calcium currents<sup>17</sup> in their dendrites but do not contain D-CaBP. However, an important electrophysiological difference between CA1 and CA3 neurones is that although calcium currents induce bursts of spikes in both CA1 and CA3 pyramidal dendrites, in CA3 pyramids these bursts propagate to the soma, whereas in CA1 a dendritic burst produces only a single somatic spike. Perhaps D-CaBP, by regulating intracellular calcium levels, is limiting the temporal and spatial spread of calcium currents, thereby preventing or modifying bursting activity. It is thus possible that the presence of D-CaBP in a neurone is indicative of voltagedependent calcium currents in that cell; however, this idea must be tested by intracellular recording from at least some of the cell types listed in Table 1. The pyramids of CA3, however, make it clear that calcium spikes need not imply the presence of D-CaBP. This situation may be quite common because D-CaBP was not found in dorsal root ganglia, although some ganglion cells can produce calcium spikes18

If cells containing CaBP are to be regarded as target cells for 1,25-(OH)<sub>2</sub>D<sub>3</sub>, they should also contain this hormone in addition to one of the products of its action. Combining the results of this study with those 9,19 on the autoradiographic localization of 1,25-(OH)<sub>2</sub>D<sub>3</sub>, which used the atlas of Konig and Klippel<sup>26</sup> following sites in brain contain both the hormone and D-CaBP: amygdala, nucleus pariventricularis, nucleus parataenialis in the thalamus and some neurones in the nucleus spinalis caudalis of the nervous trigeminus. Although low uptake of labelled

Table 1 Localization of D-CaBP in brain regions of rat and chicken

Region in chicken	D-CaBP concentration		Region in rat	D-CaBP concentration
Medulia and pons				
Substantia gelatinosa trigemini	+	386	Nucleus trigemini spinalis	++
Nucleus solitarius	+++	100	Nucleus solitarius	++
Inferior olive	++	100	Inferior olive	++
Nucleus magnocellularis	++	-	Ventral cochlear nucleus	+
Nucleus subtrigeminus	++		Dorsal cochlear nucleus (scattered cells)	
Descending vestibular nucleus (scattered cells)	+++		Nucleus of trapezoid body	++
Nucleus tangientielis	++		Dorsal nucleus of the lateral lemniscus (scattered cells)	+
Nucleus laminaris	++		Dorsal nucleus of the lateral lemniscus (scattered cells)	++
Principal trigeminal nucleus				
Lateral reticular formation (scattered cells)	+			
	+			
Nucleus ventralis lemniscus lateralis	+++			
Cerebellum Cerebellum				
Purkinje cells	+++	-	Purkinje cells	+++
•			Turkinje cens	777
fesencephalon				
Tectum (scattered cells)	++	300	Superior colliculus (scattered cells)	++
Area pretectalis (scattered cells)	++	#	Pretectal area (scattered cells)	++
Ventral tegmental area of Tsai (scattered cells)	+++	<b>86</b>	Ventral tegmental area of Tsai (scattered cells)	++
Nucleus linearis caudalis	+		Central tegmental nucleus (scattered cells)	++
Nucleus intercollicularis (scattered cells)	+		Lateral tegmental nucleus (scattered cells)	++
Nucleus subceruleus ventralis (scattered cells)	+		Carried Annual Committee Annual Committee Comm	
Nucleus mesencephalicus nervi trigemini	++			
Nucleus mesencephalicus profundus (scattered cells)	+++			
Nucleus papillioformis (scattered cells)	++			
Nucleus ectomammilaris	+			
Nucleus isthmi magnocellularis	++			
rucicus istinui magnocenularis	++			
Diencephalon				
Medial habenula	+++	195	Medial habenula	+++
Spiriformis medialis	+		Nucleus lateralis thalami, pars posterior (scattered cells)	+
Spiriformis medialis	++		Nucleus ventralis thalami (scattered cells)	, +
Nucleus rotundus	+		Nucleus ventralis thalami, pars medialis	++
Nucleus triangularis	++		Nucleus reuniens	+++
Nucleus subhabenularis lateralis (scattered cells)	++		Rhomboid nucleus	
				++
Nucleus ventrolateralis thalami (scattered cells)	+++		Mediodorsal nucleus (scattered cells)	+
Area hypothalami posterioris (scattered cells)	++		Paraventricular nucleus of thalamus	++
Nucleus medialis hypothalami posterioris (scattered cells)	++		Parataemial nucleus of thalamus	++
Nucleus mamillaris lateralis (scattered cells)	++		Zona incerta	++
Stratum cellulare internum (scattered cells)	++		Subthalamic nucleus	++
Nucleus paraventricularis magnocellularis	+++		Lateral mammillary nucleus	+
			Medial mammillary nucleus	++
			Supra mammillary nucleus	+
			Dorsal premammillary nucleus (scattered cells)	+
			Posterior hypothalamic nucleus (scattered cells)	++
			Dorsomedial hypothalamic nucleus	+
			Area hypothalamica anterior (scattered cells)	+
			Perifornical neurones and neurones within medial forebrain bundle	++
			Supraoptic nucleus	+
			Paraventricular nucleus	++
			Arcuate nucleus (scattered cells)	+++
'alaman hatan				
elencephalon Paleostriatum orimitiuum	.1.		I staral cantal nuclaus	
Paleostriatum primitivum	+		Lateral septal nucleus	+
Neostriatum and hyperstriatum (scattered cells, small	+++		Hippocampus (granule cells of dentate gyrus and pyramidal	++
nucleus)			cells of CAI)	
			Amygdala (scattered cells)	++
			Pyriform cortex, deep cells	++
			Entorhinal cortex, deep cells	++
			Olfactory cortex, deep cells	++
Olfactory bulb, periglomerular cells	+		Olfactory bulb, periglomerular cells	+++

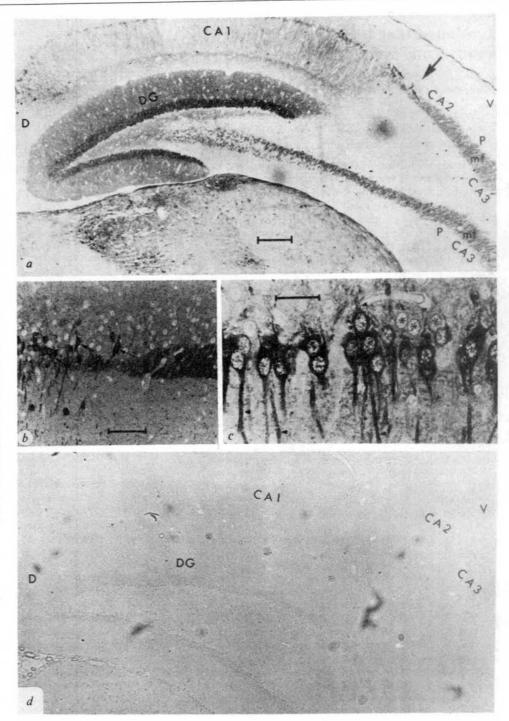
Monospecific antiserum was raised against chick duodenal D-CaBP purified to electrophoretic homogeneity by the original method of D-CaBP isolation<sup>24</sup>. Brain sections (7 µm) were prepared from paraffin-embedded material after fixation with Carnoy (for rationale of fixation and further details of the technique, see ref. 8). Antiserum was used at 1:10 dilution, while goat anti-rabbit y-globulin conjugated with peroxidase (Miles) was diluted as 1:50. Treatment with 3,3'-diaminobenzidine was for 15 min. Control sections were stained with normal rabbit serum and with supernatant from antigen and antiserum mixed in proportions of equivalence point. Other sections were stained with cresyl violet. The concentration of D-CaBP was ascertained only visually and is expressed as +++, ++ and +. +++ Implies a dense reaction in the somata which was also easily visible in the dendrites, axons and their terminals<sup>8</sup>, while + indicates a lesser reaction in the perikaryon which may extend into the processes but was not easily detectable there. ++ Implies an intermediate situation where the reaction product was quite dark in the somata and was easily detectable in the processes but only in their proximal parts. Unless otherwise mentioned (for example, scattered cells), all neurones in each nucleus stained for D-CaBP. Those regions for which there is good comparative anatomical evidence for homology are marked = whereas regions which are not known to be homologous are listed separately. Nomenclature for the brains is drawn primarily from the atlases of Karten and Hodos<sup>25</sup>, and Pellegrino et al.<sup>26</sup>.

hormone has been demonstrated in the spinal cord, no D-CaBP was detected.

There is therefore an encouraging correlation of identity of the target cells by the two techniques, for it must be remembered that all D-CaBP-containing cells, because of the dependency of this protein on vitamin D in the intestine, must be target cells, whereas all target cells need not be positive for D-CaBP, as the synthesis of this protein may not be the response of such cells to vitamin D stimulation. However, D-CaBP is present in many

other organs<sup>5,6</sup> and in some cases the cell types have been identified<sup>8,21</sup>. Evidence for the view that a wide range of tissues have target cells for 1,25-(OH)<sub>2</sub>D<sub>3</sub> is provided by the autoradiographic localization of the hormone in cells which also contain D-CaBP<sup>9,19,22</sup>, only a few of which occur in the central nervous system. Initial studies with epithelial tissues such as the intestine and shell gland have connected the function of D-CaBP with transcellular calcium transport, as seen in calcium absorption or egg shell formation<sup>23</sup>. This view is clearly not

Fig. 1 a-c, Transverse sections of rat hippocampus, stained with antiserum. In power view (a) the dark reaction product indicating the presence of D-CaBP is seen in discrete sites. The blank areas serve as internal controls. For orientation, D and V denote dorsal and ventral sides of the brain. The D-CaBP is seen in the granule cells of the dentate gyrus (DG) and their mossy fibre efferents (mf). Pyramidal cells (small arrows) within CA1 are also positive. b Shows a higher magnification of the area about the large arrow in a, illustrating the abrupt transition between CA1 and CA2. Note that whereas pyramidal cells of CA1 (small arrows) are positive, pyramidal cells of CA2 as well as CA3 (P in a) and their Schaeffer collaterals within CA1 are devoid of D-CaBP. c, A further higher magnification view of CA1 shows the reaction product in both apical and basilar dendrites (arrow heads) of CA1 pyramidal cells. Note that some neurones do not show any reaction product (asterisk). It is not known whether these are pyramidal cells or another type of cell (for example, basket cells). Scale bars in a, b and c represent 300, 100 and 40 µm respectively. d, Control, similar to a but from section stained with normal rabbit serum.



supported by the presence of D-CaBP in so many cell types uninvolved in such processes. It may be that D-CaBP is involved in more than one physiological function, in which case it might have a single molecular action which has been incorporated into

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a variety of processes. The latter almost certainly involve the membrane transport of calcium but whether the protein is involved at this point or in the subsequent need to regulate intracellular calcium levels awaits further study.

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# Evidence that types I and II interferons have different receptors

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Interferons (IFNs) are a class of proteins, secreted by animal cells in response to various inducers1, which confer resistance to viral infections and are designated according to their cellular origin or to the inducing agent. Viruses induce type I interferon, subdivided into  $\alpha$ -interferon, produced by leukocytes (Le) or lymphoblastoid (Ly) cells, and  $\beta$ -interferon, produced by fibroblasts1. Mitogens and antigenic stimuli induce in lymphocytes type II immune IFN-y. Interferons seem to bind to specific receptors<sup>2</sup> to elicit a variety of cellular responses<sup>3</sup>; several early studies provided indirect evidence for such binding4-6. Direct evidence for specific interferon receptors was presented by Aguet<sup>7</sup>, who showed that biologically active <sup>125</sup>I-labelled mouse (Mu)IFN binds to sensitive L1210-S cells, but not to interferonresistant L1210-R cells8. The main obstacle in carrying out such studies is the availability of pure interferon. Recently, Maeda et constructed plasmids containing human interferon sequences. The plasmid p104 was used as a probe to isolate the coding region of a human interferon (IFLrA), which was expressed in Escherichia coli<sup>16</sup> and purified with monoclonal antibodies<sup>11</sup>. This interferon, designated HuIFN- $\alpha$ A, was labelled with 125 I for binding assays on human cells. We have determined the specificity of different HulFNs for the cellular sites which bind HuIFN-αA and show here that HuIFN-γ does not compete for binding, whereas all type I HuIFNs do.

The HuIFN- $\alpha$ A was iodinated in conditions designed to minimize the concentration of  $H_2O_2$  in the reaction (Fig. 1). The high stability of the interferon allowed prolonged incubation and resulted in retention of biological activity. The <sup>125</sup>I-labelled HuIFN- $\alpha$ A had a specific activity of ~50 mCi per mg and an antiviral titre in different experiments of 50-75% of the unreacted interferon, when assayed within a week after iodination. The HuIFN- $\alpha$ A was iodinated in ~15% of the tyrosine residues, as determined from the specific activity of <sup>125</sup>I and the recovery of labelled protein. As there are four tyrosines per HuIFN- $\alpha$ A molecule<sup>12</sup>, at most 60% of the molecules were mono-iodinated. We have as yet no information on the proportion of molecules having different degrees of iodination.

The product of these iodination reactions showed a major molecular weight  $(M_{\rm r})$  component of  $\sim 20,000$  (as calculated from the DNA sequence  $^{12}$ , HuIFN- $\alpha$ A has a  $M_{\rm r}$  of 19,390) and traces of other components, when analysed by gel electrophoresis (Fig. 1). This preparation was further fractionated by chromatography on Sephadex G-75 and the fractions analysed by gel electrophoresis and for antiviral activity (Fig. 1). A single peak of antiviral activity was eluted, corresponding to the major peak of radioactive protein. This peak was well separated from iodinated bovine serum albumin (BSA), which represented the major protein contaminant. Some carrier BSA was presumably iodinated when added after inhibiting the reaction with NaN<sub>3</sub> (see Fig. 1 legend). The fractions containing  $^{125}$ I-HuIFN- $\alpha$ A were combined and used in subsequent experiments.

We investigated the interaction of  $^{125}\text{I-HuIFN-}\alpha\text{A}$  with cellular binding sites by incubating it with suspensions of human cells in tissue culture. At the end of the incubation, the cells were centrifuged through two sucrose layers. and the radioactivity sedimenting with the cell pellet was measured (Table 1). Greater binding was obtained with Daudi cells than with the other lymphoblastoid cells tested or with HeLa cells. Therefore, we used Daudi cells in all further experiments. Even with these cells, relatively small amounts of  $^{125}\text{I-HuIFN-}\alpha\text{A}$  were bound and the assays were carried out with 4–10 ml of cell suspension.

Binding was extremely reproducible: each preparation of labelled interferon gave essentially identical results when tested in standard conditions for several weeks, despite some decline in antiviral activity.

The time course of binding at 0 °C and 37 °C is shown in Fig. 2a. There was very little binding at 0°C, whereas at 37°C substantial binding occurred which reached a plateau after ~1 h. All subsequent experiments were carried out at 37 °C, the temperature of the cell cultures. 125I-HuIFN-αA binding approached saturation when increasing concentrations were incubated with a standard cell aliquot; a Scatchard analysis of these data gave an apparent single affinity constant of  $7 \times$ 10° M<sup>-1</sup> (Fig. 2b). This binding was abolished when large amounts of HuIFN- $\alpha$ (Le) were included in the incubations (Fig. 2b). These results indicate that the binding involves specific cellular sites. The number of binding sites per cell, calculated from the intercept with the abscissa in the Scatchard plot14, is -5,000. These observations are consistent with the binding of <sup>125</sup>I-HuIFN- $\alpha$ A to cellular receptors, although the identification of a specific receptor would require its characterization as a macromolecular complex with interferon or its purification. For

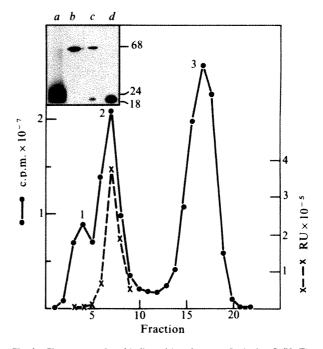


Fig. 1 Chromatography of iodinated interferon on Sephadex G-75. The HuIFN-αA was purified by Dr Sidney Pestka (Roche Institute of Molecular Biology) on an affinity column of monoclonal antibodies to a specific activity of  $2\times10^8$  U per mg. It was iodinated in a reaction containing in 0.1 ml: 2.2  $\mu$ g HuIFN- $\alpha$ A, 12.5  $\mu$ M Na<sup>125</sup>I (2.5 mCi,  $\sim$ 2,000 Ci mmol<sup>-1</sup>), 10  $\mu$ l of hydrated Enzymobead iodination reagent (Bio-Rad) containing immobilized glucose oxidase and lactoperoxidase, 0.3%  $\beta$ -D-glucose and phosphate-buffered saline (PBS) pH 7.0. The reaction was stopped after 24 h by the addition of 0.2 M NaN3. After 15 min, 0.2 mg of BSA in 0.1 ml PBS was added and the sample applied to a  $2.5\times0.7\,\mathrm{cm}$  column of Sephadex G-10 equilibrated with PBS containing 1 mg ml $^{-1}$  BSA (buffer A). This column was washed with 1 ml of buffer A and the cluate applied to a  $25\times0.7\,\mathrm{cm}$ column of Sephadex G-75 equilibrated with buffer A. The column was eluted at a flow rate of 6 ml h<sup>-1</sup> and 0.44-ml fractions were collected. All these operations were carried out at room temperature. The column fractions were stored at 4 °C and analysed for radioactivity ( ) and antiviral activity (×). The latter was measured by the protection of monolayers of the human lung carcinoma cell line A549 from infection with encephalomy-ocarditis virus. Monolayers in multi-well plates were infected with  $4\times10^5$ plaque-forming units of virus and the cytopathic effect quantitated by a viral dye uptake method<sup>22</sup>. The HuIFN- $\beta$  standard (G-023-902-527, obtained from NIH) was used as a reference and the antiviral titre is expressed in NIH room NH) was used as a reference and the ainth at other is expressed in NH? reference units (RU). Column fractions were also analysed by electrophoresis on 15% polyacrylamide slab gels<sup>23</sup>. The autoradiographs are shown in the top left corner: a, unfractionated <sup>125</sup>I-HuIFN- $\alpha$ A; b, peak 1; c, fraction 5; d, peak 2. About 10,000 c.p.m. of each fraction and 100,000 c.p.m. of unfractionated interferon were analysed. The molecular probability in the exercise of protein standards run in applied is indicated at the weight (in thousands) of protein standards run in parallel is indicated at the right-hand side: 68, BSA; 24, trypsinogen; and 18,  $\beta$ -lactoglobulin. Peak 3 is free  $^{125}I^-$ .

Table 1 Binding of <sup>125</sup>I-labelled interferon to human cells

Cell line	125I-HuIFN-αA bound (c.p.m.)
Daudi	880
Raji	270
Molt-4	260
HeLa	310

The lymphoblastoid cells were grown in stationary cultures or in roller bottles in Dulbecco's medium with 10% horse serum; HeLa cells were grown in spinner cultures in Eagle's medium supplemented with 7.5% calf serum. Each assay contained in 5 ml of culture medium:  $2.5 \times 10^6$  cells and 2 ng of <sup>125</sup>I-HuIFN- $\alpha$ A ( $\sim$ 60,000 c.p.m.). After 1 h at 37 °C the samples were applied over two 4-ml layers of ice-cold 5 and 10% sucrose in buffer A and centrifuged at 20,000g for 2 min<sup>13</sup>. The pellet obtained was counted in a  $\gamma$ -counter.

convenience, the cellular binding sites for HuIFN- $\alpha A$  are referred to as receptors.

The observation that unlabelled interferon abolished the binding of <sup>125</sup>I-HuIFN-αA led us to assay different interferons in competition experiments (Fig. 3). The HulFNs used differed widely in purity, and antiviral activities had been measured in different laboratories. We determined the antiviral titre using the standard assay described in Fig. 1 legend. The competition with unlabelled HuIFN-αA could be superimposed on that obtained with HuIFN- $\alpha(Lv)$ , whereas HuIFN- $\alpha(Le)$  competed slightly less effectively (Fig. 3a). HuIFN- $\beta$  preparations of quite different purity gave superimposable competition curves, indicating that other proteins present in the preparations do not interfere in this assay (Fig. 3b). Finally, HuIFN- $\gamma$  and MuIFNβ, which is inactive on human cells 15, competed only poorly, if at all (Fig. 3b). The competition with different HuIFN- $\beta$  samples could not be explained by the presence of contaminating HuIFN- $\alpha$  in the preparations. This was established by preincubating HuIFN- $\beta$  and HuIFN- $\alpha$ (Le) with an appropriate amount of anti-HulFN- $\alpha$ (Le) antiserum, which does not significantly cross-react with  $HuIFN-\beta$ , as previously described<sup>16</sup>. This resulted in decreased competition of the HuIFN- $\alpha$ (Le), but had no effect on HuIFN- $\beta$  competition (see Fig. 3 legend). It seems, therefore, that all type I HuIFNs compete with <sup>125</sup>I-HuIFN-aA for binding to the same receptors.

These results show that HuIFN- $\gamma$  does not bind with high affinity to HuIFN- $\alpha$ A receptors and indicate that type II interferon may interact with different receptors. Ankel *et al.*<sup>17</sup> proposed that there are two distinct classes of interferon-binding

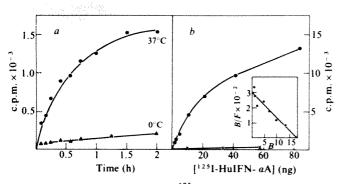


Fig. 2 Time course of binding of  $^{125}$ I-labelled interferon (a) and saturation of binding sites (b). For each time point in a,  $8\times10^6$  Daudi cells were incubated in 4 ml at the indicated temperature with 1 ng  $^{125}$ I-HuIFN- $\alpha$ A, as described in Table 1. In b,  $5\times10^6$  Daudi cells in 10 ml were incubated at 37 °C for 90 min with the indicated amount of  $^{125}$ I-HuIFN- $\alpha$ A; the c.p.m. bound per assay are indicated ( $\blacksquare$ ). Binding in the presence of  $4\times10^3$  units of HuIFN- $\alpha$ (Le) per ng HuIFN- $\alpha$ A was also determined (×). Inset shows a Scatchard plot of the data in b; B and F are concentrations of bound and free  $^{125}$ I-HuIFN- $\alpha$ A (c.p.m.× $10^{-3}$ ). The samples were centrifuged through two sucrose layers of 9 ml each, as described in Table 1 legend.

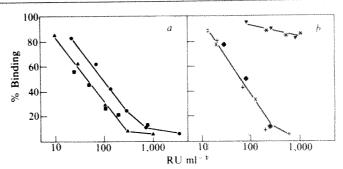


Fig. 3 Competition of different interferons with <sup>125</sup>I-HuIFN-αA for binding to Daudi cells. Incubations were as described in Fig. 2a, with the addition of the indicated concentrations of the following interferons: HuIFN-α(Le) (•; from Dr K. Cantell), HuIFN-α(Ly) (A; from Dr C. B. Anfinsen), unlabelled HuIFN-αA (■), highly purified (>10<sup>8</sup> RU per mg) HuIFN-β (\*); from Dr E. Knight Jr), two less purified samples of HuIFN- $\beta$  (×; from Dr J. Horoscewicz, 106 RU per mg; and +; from the Interferon Working Group of the NCI, 3×10<sup>5</sup> RU per mg). HuIFN-y (♥), purified as described elsewhere<sup>24</sup>, was given by Dr J. Vilček and Dr Y. K. Yip, and MuIFN-β (\*) was a gift from Dr Peter Lengyel (this preparation was purified to  $> 10^8$  RU per mg (ref. 15) and was neutralized using anti-MuIFN- $\beta$  antiserum<sup>25</sup> in Dr Lengyel's laboratory). The results are plotted as % binding relative to an assay with no added competitor. As a control for the specificity of the competition, 2,000 RU of HuIFN-α(Le) or HuIFN-β in 0.1 ml were incubated with 20 µl anti-HuIFN- $\alpha$ (Le) antiserum at 37 °C for 30 min and then overnight at 0 °C, as described elsewhere <sup>16</sup>. These samples were tested in competition assays. The binding with HulFN- $\beta$  was 11%, unchanged relative to a sample not treated with antiserum, whereas that with HuIFN- $\alpha$ (Le) increased from 10 to 34%.

sites in mouse cells, each specific for one interferon type, on the basis of experiments with L1210-R cells selected for resistance to Type I MuIFN<sup>8</sup>; these cells responded to the antiviral and anti-growth activities of type II interferon 17. Moreover, gangliosides, which may be part of type I interferon receptors 5, inhibit the activity of type I but not that of type II interferon 17. Distinct receptors for the two interferons may account for the different pathways of induction of the 2'5'-oligo(A) polymerase elsewhere reported 18 and possibly for the potentiation of activity of one interferon type by simultaneous treatment with the other 19. Interferons of the same type, however, do not potentiate each other but show additive effects 16, as expected if they interact with the same receptor.

In these studies we measured interferon binding at 37 °C. At this temperature interferon may interact with cellular receptors in a complex way, possibly as a result of a series of metabolic events. This was suggested by the observations of Friedman<sup>4</sup>, who treated chick fibroblasts with interferon at 4 °C. Digestion with trypsin at this temperature inhibited the development of the antiviral state when the cells were shifted to 37 °C. A short incubation at 37 °C, however, apparently rendered the interferon trypsin resistant. This led Friedman<sup>4</sup> to postulate that interferon binding at 4 °C involves cell-surface sites accessible to trypsin. Aguet<sup>7</sup> previously reported that binding of <sup>125</sup>I-labelled MuIFN to L1210-S cells is markedly higher at 37 °C than at 4 °C; in this study an apparent dissociation constant  $(K_d)$  of  $1-2\times10^{-11}$  M was estimated from the interferon concentration required to reach half-saturation of binding sites. From our data we calculate a  $K_d$  of  $\sim 1.5 \times 10^{-10}$  M. These  $K_d$  values may reflect the different specific activities of the interferons:  $1 \times 10^9$  U per mg protein estimated by Aguet<sup>7</sup> for the MuIFN and  $2 \times 10^8$  U per mg for the HuIFN- $\alpha$ A. The biological significance of these differences in specific activity remains to be established.

Recent advances, in the study of many polypeptide hormones have provided guidelines for investigating their interaction with cellular receptors<sup>20,21</sup>. Interferons may be regarded as polypeptide hormones because of their role in communicating from cell to cell a specific set of instructions, which leads to a wide variety of effects<sup>3</sup>. These studies provide an initial characterization of a HuIFN-receptor interaction, and are potentially

useful in studying the role of interferons as regulators of cellular

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#### Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells

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A recent nationwide survey of the lymphocyte subpopulations of leukaemia and lymphoma in Japan has disclosed a high incidence of adult T-cell leukaemia (ATL)1. One of the striking features of this disease is the clustering of patients in the southwestern part of Japan 1,2. We have established a continuous culture line of leukaemic T cells from a patient with ATL 3.4. This cell line, MT-1, was found to carry type C virus particles and ATL-associated antigens (ATLA) which specifically reacted with sera from all ATL patients and about 25% of healthy adults in the endemic area5. We report here that by co-culture of ATL cells from a female patient and normal human cord leukocytes from a male infant, a T-cell line of cord leukocyte origin was established. Numerous type C virus particles as well as ATLA were detected in this cell line.

A 45-yr-old woman was admitted to the hospital with generalized lymphadenopathy and hepatosplenomegaly. Her blood leukocyte count was 207,300 per mm3, with 93% abnormal lymphoid cells having indented or lobulated nuclei. Most of these cells formed spontaneous sheep erythrocyte rosettes. Peripheral leukocytes separated by Ficoll-Conray gradient centrifugation were cultured in 35-mm Petri dishes (3 ml per dish) with RPMI 1640 medium supplemented with 10% human cord serum, 10% fetal calf serum and antibiotics. The initial cell density was adjusted to 5×106 per ml. The cultures were incubated at 37 °C in a humidified 7.5% CO2 atmosphere and fed twice a week. After 2 months of culture, the number of cells decreased considerably and the cells were co-cultured with umbilical cord leukocytes ( $1 \times 10^6$  per ml) from a male infant, with the hope of stimulating the growth of ATL cells. After 2 months of co-culture, a lymphoid cell line was established that was transferable every 3-5 days. This cell line, MT-2, grew in suspension forming clumps of cells and has been maintained in continuous culture for 1.5 yr. No T-cell growth factor was used. Unexpectedly, cytogenetic studies on MT-2 showed a normal male karyotype and its cord leukocyte origin was determined. MT-2 cells expressed complement receptors and Ia antigens in addition to having sheep erythrocyte receptors and T-cell membrane antigens. The cultured cells were negative for Epstein-Barr virus nuclear antigen. The establishment and characteristics of this cell line have been briefly reported elsewhere6

The acetone-fixed cells were examined for ATLA by indirect immunofluorescence using ATL patients' sera and fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG as previously described<sup>5</sup>. The MT-2 line gave a positive reaction with three reference sera from ATL patients but not with three negative control sera. Almost 100% of cells showed brilliant staining in the periphery of the cytoplasm at a serum dilution of 1:10 (Fig. 1). Unfixed MT-2 cells were similarly stained by the indirect immunofluorescence method and almost all the cells exhibited surface membrane fluorescence with the positive reference sera. These reference sera, however, did not react with various human haematopoietic cell lines, including six T-cell lines (HPB-ALT, HPB-ALL, TALL-1, RPMI 8402, Molt-4 and CCRF-CEM), four B-cell lines (Raji, Wil-2, TL-1 and BALL-1) and four non-T non-B cell lines (HL-60, NALL-1, HPB-NULL and K-562). Of these, TALL-1, BALL-1 and NALL-1 are acute lymphoblastic leukaemia cell lines established by us and maintained in our laboratory in the same conditions as used for MT-2. This would exclude the possibility that some serum component(s) or other exogenous products in the culture medium elicited the immunofluorescent reaction. Thin-section electron microscopy of MT-2 cells revealed numerous type C virus particles in the extracellular spaces (Fig. 2). The virus particles which were predominantly mature were between 85 and 145 nm in diameter. The mature virus particles consisted of an electron-dense nucleoid and an outer membrane. The immature virus particles were doughnutshaped with an electron-luscent centre. Virus particles budding from the cell membrane were rarely observed.

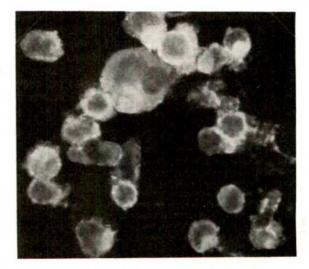


Fig. 1 Immunofluorescence micrograph of acetone-fixed MT-2 cells showing ATLA in the cytoplasm with brighter fluorescence in the periphery. All the cells, including giant cells, are fluorescent. The cells were first reacted with serum from an ATL patient and then incubated with FITC-conjugated goat anti-human IgG. ×750.

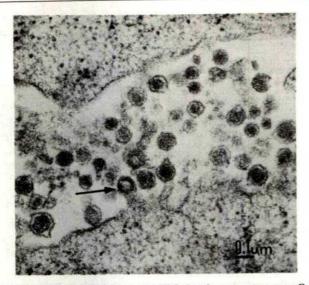


Fig. 2 Electron micrograph of MT-2 showing numerous type C virus particles in the extracellular space. One particle is budding from the cell membrane (arrow). The cells were fixed in 3% glutaraldehyde and 1% osmium tetroxide and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate. ×59,000.

Fresh ATL cells from the patients do not express ATLA; this antigen expression and type C virus production occur spontaneously after several days of in vitro culture (Y.H., unpublished observation). This is consistent with persistent association of ATLA and type C virus particles in a cultured ATL cell line MT-1 (ref. 5). It is postulated, therefore, that during co-culture of ATL cells and normal human cord leukocytes, a type C virus or its genome was transmitted from ATL cells to cord T cells by extracellular or cell-to-eell infection and that the infected T cells were transformed into the virus-producer cell line MT-2. The presence of exclusively ATLA-positive cells and numerous virus particles in MT-2 is indeed significant because MT-1 contained only a few per cent of ATLA-positive cells and rare virus particles5. Consistent with the present report, we have recently characterized another T-cell line derived from cord blood lymphocytes by co-cultivation with ATL cells from a different patient. This cell line also harbours abundant type C virus particles and ATLA (manuscript in preparation). Contamination of our cells by animal oncoviruses is unlikely, as neither these viruses nor murine cells have been handled in our laboratory for several years. According to Barbacid et al.7, human sera recognized glycoproteins of animal oncoviruses grown in rodent or carnivore cells but not those of the same viruses grown in

The present results strongly suggest that the type C virus we have detected in our cell lines is aetiologically related to ATL. Recently, Poiesz et al.8 also isolated a type C retrovirus in a T-cell line derived from a patient with cutaneous T-cell lymphoma (mycosis fungoides). Thus, these independent observations in the United States and Japan lead us to think that at least certain T-cell malignancies may be caused by a closely related, if not identical, viral agent.

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#### Rous sarcoma virus transforming protein, p60src, expressed in E. coli, functions as a protein kinase

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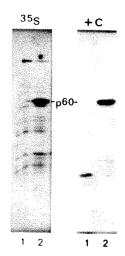
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The product of the Rous sarcoma virus (RSV) transforming gene, src, is a phosphoprotein of molecular weight  $(M_r)$  60,000 (pp60src)1-3 that is responsible for cell transformation as well as fibrosarcoma formation in a variety of animals<sup>4,5</sup>. Several experiments suggest that pp60<sup>arc</sup> is a protein kinase<sup>6-10</sup> with the unusual capacity of phosphorylating tyrosine residues 11,12, but additional evidence would be of value. Earlier studies had suggested that bacteria were unable to carry out protein phosphorylation13, although more recent findings indicate that distinct protein kinases and phosphatases are present in Salmonella typhimurium14. Despite this observation, because of the evolutionary distance, the enzymatic activities observed in bacteria are likely to be carried out by proteins unrelated to those expressed in eukaryotic cells. Thus, to study the RSV transforming protein synthesized in the absence of the variety of protein kinases expressed in normal host cells (for reviews see refs 15, 16), we have now constructed plasmids that express p60src in Escherichia coli. Analysis of p60src produced in E. coli and selected by immunoaffinity chromatography, shows that it has the capacity to phosphorylate proteins at tyrosine residues; this activity is specifically inhibited by anti-p60src IgG. Extracts from E. coli carrying identical plasmids but lacking the src gene yield no detectable enzyme activity. These data, taken with those previously published 9,10,17, lead to the conclusion that the RSV src gene encodes a protein kinase.

Bacteria carrying recombinant molecular clones were radiolabelled in culture with 35SO4- and the cellular extracts subjected to immunoaffinity column chromatography as described in Fig. 1 legend. The presence of p60 among the proteins eluted from the columns was confirmed by phosphorylation of the preparations in vitro with the catalytic subunit of the cyclic AMP-dependent protein kinase (CAT), a previously characterized post-translational modification of pp60src (ref. 18). As shown in Fig. 1, lysates prepared from bacteria carrying a *lac-src* fusion gene in pBR325 yielded both a  $^{35}$ S-labelled protein of  $M_r$  60,000 and a protein of the same molecular weight that could be phosphorylated by CAT, whereas lysates prepared in exactly the same way from cells carrying only lac UV5 DNA in pBR325 yielded no detectable protein of a similar nature.

To determine whether p60src prepared from bacteria as described above functioned in a manner similar to pp60src prepared from eukaryotic cells, protein kinase reactions were carried out and the results are presented in Fig. 2. To reduce the possibility that any activity detected originated in the protein substrates or the IgG, these were incubated at 60 °C for 5 min before use. The results show that the extract prepared from the lac UV5-pBR325 bacteria yielded no detectable phosphotransferase activity when assayed alone or with  $\alpha$  and  $\beta$ tubulin or casein as potential substrates (Fig. 2, tracks 1, 2, 3 [lac]). In contrast, an equal quantity of extract prepared from the lac-src bacteria yielded readily demonstrable phosphorylation of  $\alpha$  and  $\beta$  tubulin and case in (Fig. 2, tracks 1, 2, 3 [lac-src]). These proteins have been shown to be efficiently phosphorylated by pp60<sup>src</sup> prepared from eukaryotic cells <sup>10,12,17</sup>. Moreover, the phosphorylation of casein was inhibited by the prior addition of anti-p60src IgG but not IgG from a non-immune rabbit (tracks 4, 5). Note that anti-p60src

Fig. 1 Polyacrylamide gel electrophoretic analysis of bacterial extracts after immunoaffinity chromatography. The lac UV5 promoter-operator 203-base pair (bp) fragment was constructed by F. Fuller and its use as a 'portable promoter' was described previously21. The DNA fragment (obtained from David L. Hare and John R. Sadler of this institution) contains an EcoRI site 65 bp from the start point of transcription and encodes nine amino acids of the *lacZ* gene. By the use of *EcoRI* linkers this promoter was fused to the 5' end of the *src* gene previously cloned from RSV-infected cells<sup>22</sup>. *E. coli* expressing p60 was detected by immunoprecipitation of <sup>35</sup>S-labelled extracts from cells containing the *lac* UV5 promoter-operator as described previously<sup>20</sup>, and one of these clones was used for the studies described here. E. coli carrying the lac UV5 promoter in pBR325 in the same orientation as in the src-containing clone served as a control for the expression of p60. DNA sequence analysis and restriction nuclease mapping of the cloned DNA indicated that the primary translation product under control of the lac promoter should contain the entire src gene product plus 11 additional amino acids at the NH2 terminus encoded by the lacZ gene and the linkers used to construct the plasmid. Peptide mapping of the p60 produced in E. coli supported this interpretation. Bacteria were grown in M9 minimal medium (6 g Na<sub>2</sub>HPO<sub>4</sub>, 3 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g NaCl, 1 g NH<sub>4</sub>Cl per 1) containing 0.8% glucose, 0.4% casamino acids, 2 μg ml<sup>-1</sup> thiamine, 40 μM MgSO<sub>4</sub>, 0.1 mM CaCl<sub>2</sub>, 1 mM isopropyl-β-D-thiogalactopyranoside and 2.5 μCi ml<sup>-1</sup> H<sub>2</sub><sup>35</sup>SO<sub>4</sub> (NEN) to an A<sub>550</sub> of 1.5, collected by centrifugation, washed once in phosphate-buffered saline and frozen quickly in liquid nitrogen. Frozen pellets corresponding to about 11 of cells were prepared for immunoaffinity chromatography by suspension in 14 ml of 10 mM Tris, 20% sucrose and 1.5 mg ml<sup>-1</sup> lysozyme. After 5 min at 4 °C DNase was added to a final concentration of  $60 \mu g ml^{-1}$  and incubation continued at 4 °C for another 5 min. The lysate was brought to a final volume of 20 ml with detergent-containing buffer at a final concentration of 1% NP40, 0.5% sodium deoxycholate, 150 mM NaCl, 5 mM dithiothreitol, 10 mM Tris pH 7.2. Lysates were clarified at 10,000g for 30 min; the protein concentration at this point was about 10 mg ml<sup>-1</sup>. The 10,000g supernatant was subjected to immunoaffinity chromatography as described previously<sup>9</sup>. Briefly, serum from a tumour-bearing rabbit selected to immunoprecipitate p60s<sup>rc</sup> encoded by the Prague A strain of RSV (the strain of the original source of the DNA) was used to prepare IgG and, subsequently, an immunoaffinity matrix at a concentration of 7 mg IgG per ml of gel. About 140 mg of protein was applied to a 6-ml column and washed as described previously except that the RIPA wash was omitted and the protein adsorbed to the column was eluted with 0.75 M KSCN. The elution of protein was monitored by liquid scintillation spectrometry of a sample of each fraction and the peak fractions were immediately dialysed against 50% glycerol containing 1 mM EDTA, 20 mM phosphate pH 7.2 and 5 mM



dithiothreitol followed by dialysis against 10% glycerol and again 50% glycerol in the same buffer to achieve about a 10-fold concentration of protein. Quantitation of the protein yield is not significant because the immunoaffinity columns are overloaded in the conditions used here. Polyacrylamide gel electrophoresis was carried out as described previously.  $^{35}$ S-labelled proteins were visualized by fluorography $^{23}$  and  $^{32}$ P-labelled proteins by autoradiography with the aid of Dupont Lightning Plus intensifying screens. Left panel: Proteins purified by immunoaffinity chromatography of lysates prepared from bacteria labelled in culture with  $^{35}$ SO $_{4}^{2}$ . Track 1, lac pBR325; 2, lac-src pBR325. Right panel: The preparations depicted in the left panel were incubated with CAT in the presence of 5 mM MgCl<sub>2</sub> and 1  $\mu$ M [ $\gamma$ - $^{32}$ P]ATP before analysis. Track 1, lac pBR325; 2, lac-src pBR325. The catalytic subunit of cyclic AMP-dependent protein kinase (CAT) was purified as described  $^{24}$  and was a gift of James L. Maller.

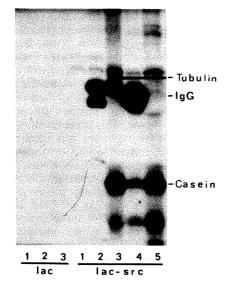


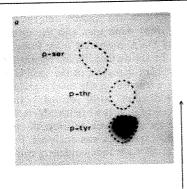
Fig. 2 Phosphorylation of various proteins by p60<sup>src</sup> partially purified from  $E.\ coli.\ 10\ \mu I\ (10\ ng)$  of the preparations described in Fig. 1 were incubated at room temperature for 10 min with the indicated additions in a total volume of 25 μl. MgCl<sub>2</sub> and  $[\gamma^{-32}P]$ ATP  $(1,000-6,000\ Ci\ mmol^{-1})$  were added to a final concentration of 5 mM and 1 μM, respectively, and incubation was continued for 30 min at room temperature. Proteins were then resolved by polyacrylamide gel electrophoresis and autoradiography. Left side (lac): Track 1, lac pBR325 alone; 2, plus  $\alpha$  and  $\beta$  tubulin; 3, plus casein. Right side (lac-src): Track 1, lac-src pBR325 alone; 2, plus  $\alpha$  and  $\beta$  tubulin; 3 plus casein; 4, plus casein and anti-p60s<sup>src</sup> IgG; 5, plus casein and non-immune IgG. Casein was present at 1 mg ml<sup>-1</sup>, the IgGs at 350 μg ml<sup>-1</sup> and tubulin at  $100\ \mu$ g ml<sup>-1</sup>. Tubulin .was prepared from rabbit brain as described previously<sup>25</sup> and was heated at 80 °C for 5 min before use.

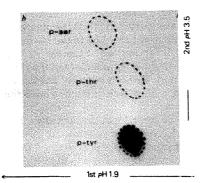
IgG is phosphorylated in the reaction, as is the case when pp60<sup>src</sup> from eukaryotic cells is similarly analysed<sup>9,10</sup>.

The protein kinase activity associated with pp60<sup>src</sup> purified from eukaryotic cells is specific for tyrosine residues in IgG<sup>11</sup> and several other protein substrates <sup>12,17</sup>. To establish further the relationship between the activity described above and that previously characterized, phosphoamino acid analysis was carried out on the products shown in Fig. 2. As seen in Fig. 3, the major amino acid phosphorylated in  $\alpha$  and  $\beta$  tubulin and in casein was tyrosine.

We describe here our preliminary characterization of the functional activity of the RSV src gene product expressed in E. coli and show that it has the capacity to phosphorylate tyrosine residues in protein substrates. Moreover, the phosphotransferase activity is inhibited by anti-p60src IgG but not non-immune IgG and results in the phosphorylation of antip60<sup>src</sup> IgG. These three characteristics, (1) immune IgG phosphorylation, (2) tyrosine specificity and (3) specific inhibition of the phosphorylation of exogenous substrates, all demonstrate the src-gene-specific nature of the enzymatic activity observed. No protein kinase activity is detectable in immunoaffinity purified preparations from identical amounts of bacteria that lack the src gene. Although the fusion protein studied here contains 11 additional amino acids (see Fig. 1 legend), we had anticipated that small changes at the amino terminus of p60src would not seriously interfere with its function because p60src shows a remarkable plasticity at the NH<sub>2</sub> terminus while still functioning to transform cells. This is most dramatically shown in studies of RSV recovered after passage of virus with partial src gene deletions through chickens, which show that the recovered viruses encode src gene products that may be 2-3,000 daltons longer or shorter at their NH2 termini but that still result in cell transformation and tumour formation19

The expression of the  $p60^{src}$ -specific protein kinase activity in  $E.\ coli$  strongly supports the previous conclusion that the enzymatic activity observed in preparations from eukaryotic cells is actually encoded in the src gene and is not the result of the co-purification of one of the many cell-encoded protein kinases expressed in RSV-infected cells. Although we estimate that the  $p60^{src}$  from  $E.\ coli$  is about 10% as active as a similar preparation from eukaryotic cells, the results described here should be regarded as qualitative because we anticipate that modification by phosphorylation with, for example, purified





**Fig. 3** Phosphoamino acid analysis of  $\alpha$  and  $\beta$  tubulin and casein phosphorylated by p60<sup>src</sup>. Phosphorylated  $\alpha$  and  $\beta$  tubulin and casein, as shown in Fig. 2, right, tracks 2 and 3, were eluted from a preparative gel, precipitated and hydrolysed in 6 M HCl at 100 °C for 3 h. The phosphoamino acids were resolved by two-dimensional electrophoresis at pH 1.9 and 3.5 and visualized by autoradiography as described previously12 Authentic phosphoamino acids were included in the sample and were visualized by ninhydrin staining. a,  $\alpha$  and  $\beta$  tubulin; b, casein.

CAT may affect the activity. The evolutionary distance of E. coli from the normal RSV hosts makes it unlikely that they would harbour protein kinases such as the cyclic AMP-dependent protein kinase known to modify p $60^{\text{src}}$  (ref. 18). We have not detected phosphorylation of p $60^{\text{src}}$  in *E. coli*<sup>20</sup> and, if additional experiments confirm that the protein is unphosphorylated in E. coli, p60src prepared in this manner should prove useful for the study of the effect of phosphorylation on its function. Similarly, analysis of the src gene product produced in E. coli should be useful for the resolution of the issue of whether pp60src has the capacity to phosphorylate itself during in vitro phosphotransferase reactions, an activity previously demonstrated in this laboratory<sup>10</sup> but not observed by others<sup>17</sup>. Additional studies will permit quantitative comparisons of the enzymatic activity of p60src of prokaryotic and eukaryotic origin.

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#### Propagation of foreign DNA in plants using cauliflower mosaic virus as vector

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Cauliflower mosaic virus (CaMV) is the best analysed member of the caulimoviruses, a group of small isometric plant viruses that contain a circular double-stranded genome (for review see ref. 1). As most plant viruses have an RNA rather than a DNA genome, caulimoviruses have attracted considerable interest due to their potential use as genetic vectors for plants. DNA purified from CaMV particles can infect plants and cause virus production when rubbed over the surface of susceptible leaves2. Moreover, the entire chromosome of several CaMV strains can be propagated through bacterial hosts by plasmid and phage  $\lambda$ vectors and has been used successfully to infect plants' Recently, Howell et al.5 reported the insertion of an 8-base pair (bp) EcoRI linker molecule into the large 'intergenic' region of cloned CaMV strain CM 4-184 DNA without impairment of infectivity. Here we report the successful propagation of foreign DNA in plants using cauliflower mosaic virus as vector. We find that the size of foreign DNA that can be successfully propagated

through virus particles has an upper limit of  $\sim 250$  base pairs. The nucleotide sequence of CaMV<sup>6.7</sup> suggests that there are extensive regions of the genome which encode protein. To insert new DNA, however, it is necessary to identify non-coding or dispensable regions where insertions will not interfere with essential functions. To identify such regions, we selected a segment of DNA which specifies a phenotype in Escherichia coli that is easily detected and selected, and which is sufficiently small so as not to exceed a possible limitation on packaging capacity of the virion. This marker DNA was then inserted into the CaMV genome cloned in an E. coli plasmid vector.

The E. coli marker chosen for these trial insertions was a 65-bp fragment derived from the lac promoter-operator region<sup>8</sup>. In a multicopy plasmid, this *lac* operator fragment leads to a lac constitutive phenotype in a suitable host due to titration of lac repressor9. We provided the fragment with an asymmetrically positioned EcoRI restriction site and added synthetic XhoI linker molecules to its ends<sup>10</sup>. XhoI linkers were chosen because the position of a naturally occurring XhoI site on the chromosome of CaMV strain 1841 is covered by a deletion in the variant strain CM 4-184 (refs 11, 12), indicating a nonessential region of the genome. The lac operator with XhoI ends could be inserted easily to test this hypothesis. As an acceptor molecule we used DNA of plasmid pCaMV10, a derivative of pBR322 carrying the complete genome of CaMV strain 1841 (refs 4, 12). The structure and orientation of two different *lac* operator inserts are given in Fig. 1.

To test whether a CaMV genome containing such inserts is infectious, the viral DNA was released from the plasmid vector by cleavage with SalI restriction endonuclease and used to infect leaves of 'Just Right' turnip plants (Brassica campestris L.). About 200 ng of DNA per leaf were sufficient to cause 2-30 local lesions on mechanically inoculated leaves and led in all cases to symptoms typical of systemic infection<sup>13</sup>. The

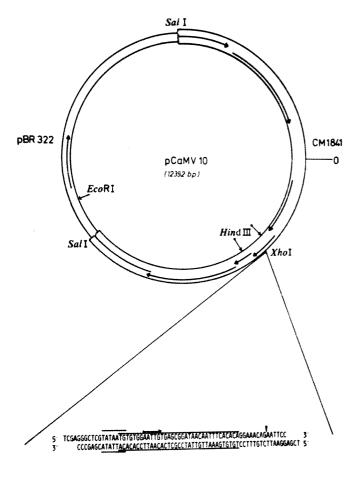


Fig. 1 Structure and orientation of the 1841-EOP2 lac operator insert at the unique XhoI site of the CaMV 1841 hybrid plasmid pCaMV10. A 55-bp HpaII-EcoRI restriction fragment from the lac UV5 promoter-operator region of E. coli in plasmid pUR108-1 (ref. 8 and U. Rüther, unpublished results) was given flush ends by incubation with E. coli Pol I Klenow fragment (Boehringer) in the presence of dATP and dTTP<sup>17</sup>. After purification by electrophoresis through an 8% polyacrylamide gel, 1 pmol of fragment was ligated to 50 pmol of Xhol linker molecules (Collaborative Research)<sup>10</sup>. This step restores the EcoRI restriction site at the promoter distal end of the fragment. Xhol restriction endonuclease digestion (restriction enzymes from New England Biolabs, Boehringer or BRL) was used to cleave off superfluous linkers and a second 8% polyacrylamide gel was used to prepare the now Xhol-ended fragment. The purified fragment was ligated to pCaMV10 DNA linearized by XhoI. Hybrid molecules were identified after transformation of E. coli CSH 51 (ref. 18) and selection of ampicillin esistant bacteria on plates containing ampicillin (50 µg ml<sup>-1</sup>) and 5-bromo-4-chloro-indolyl-B-D-galactoside (40 µg ml<sup>-1</sup>). lac constitutive bacteria produce blue colonies in these conditions<sup>18</sup>. Two different isolates were analysed further; these carry the *lac* operator in both orientations in the CaMV genome. In addition, pCaMV10-EOP1 has lost one terminal nucleotide pair at the HpaII end of the lac operator. The six open reading frames of CaMV are indicated by arrows, starting in a clockwise direction from the zero point of the map. The orientation of pBR322 relative to CaMV DNA is indicated by its unique EcoRI site and the reading frame of the B-lactamase gene. The lac operator insert of pCaMV10-EOP2 is enlarged at the bottom. The Pribnow box homology and the operator sequence are underlined. The arrow pointing to the right indicates the transcription start point in  $E.\ coli$ . The additional EcoRI site is indicated by  $\nabla$ . pCaMV10-EOP1 carries the lac operator in the opposite orientation and lacks one GC pair at the former HpaII end of the fragment.

appearance of symptoms was slightly delayed compared with infection by the parent molecules and the disease symptoms were milder. Viral DNA prepared from infected plants was analysed <sup>14</sup>. Figure 2a shows that the lac operator fragment can be recovered after XhoI digestion of the viral DNA. To determine whether only a fraction of the viral population contained the insert, four individual local lesions induced by DNA containing both types of inserts were transferred to several new plants. After systemic symptoms developed, viral DNA from each of the individual transfers was analysed. Figure 2b shows that the offspring of all eight independently transferred lesions carried the lac operator insert.

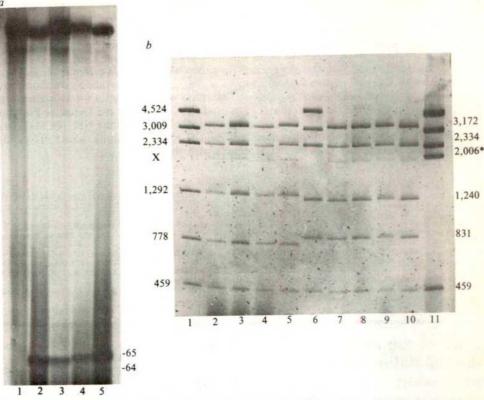
To determine whether the inserts were copied correctly during viral DNA replication we subcloned in plasmid pBR322 a HindIII restriction fragment containing the lac operator inserts at the XhoI site (Fig. 1) from either passaged CaMV DNA or from the parent plasmid used to infect the plants. DNA of these subclones was sequenced according to the Maxam-Gilbert procedure<sup>15</sup>. Figure 3 shows an example of the sequence comparison. No changes in the sequence of the two lac operator regions were detected. Regions adjacent to the site of insertion showed no sequence alteration compared with the pCaMV10 parent DNA sequence.

The stability of the inserts was checked after three successive transfers of virus derived from systemically infected tissue. All eight independent insert lines still contained the *lac* operator fragment. Some revertants occurred in the CaMV-EOP1 progeny, as indicated by the appearance of additional minor bands in the *EcoRI* restriction pattern. With CaMV-EOP2 (opposite orientation) no minor bands due to revertants could be detected. However, after five successive transfers and extended growth of the plants (2 months) the *lac* insert in both orientations was lost. In these conditions rare revertants seem to have a selective advantage over the insertion mutants which have slightly delayed growth.

To determine the maximum insertion capacity at the Xhol site, we inserted fragments of 256, 531 and 1,200 bp, respectively. The 256- and 531-bp fragments were derived from phage A DNA cleaved with SalI and XhoI (sites 44, 45 and 46 of a 1981 \( \lambda \) map update, F. R. Blattner et al., personal communication). The 1,200-bp fragment, a SalI segment of pUC5 containing the kanamycin resistance gene of Tn903 (Vieria and J. Messing, unpublished results), carries PstI sites adjacent to its SalI ends, with the kanamycin resistance gene inserted between the PstI sites by G·C homopolymer tails. Infectivity assays using CaMV DNA containing these inserts were performed as described above. After development of systemic symptoms, viral DNA was prepared from infected leaves and analysed. Figure 4 shows the EcoRI digestion pattern of CaMV DNA derived from the 256- and 531-bp insertions; only the former was propagated stably. Insertion of 531 bp gave rise to three different deletion mutants in the region of insertion. These infections were apparent in plants only after a prolonged period: 5-6 weeks after inoculation as opposed to 2 weeks normally required for systemic development. In addition, attempts to recover infectious CaMV DNA molecules containing the 1,200bp fragment were unsuccessful. Analysis of the viral DNA from plants that developed symptoms showed that the inserted fragment was lost, perhaps by recombination in the homopolymer tails as most of the isolates gained at least one additional PstI site at the insertion point.

Thus, we have provided evidence for a non-essential region in the CaMV genome. Insertion of foreign DNA from two different sources into an XhoI restriction site whithin this region does not interfere with the infectivity of the viral DNA nor with virus production. However, the stability of the 256-bp  $\lambda$  DNA insert in a systematic survey has not been compared with that of the lac EOP inserts. The instability of the 64/65-bp lac promoter-operator inserts could be due to some peculiarity of the DNA sequence itself. Note for example the similarity of the lac UV5 Pribnow box to the 'TATAA' initiation signal sequence for RNA polymerase II in eukaryotes<sup>16</sup>. Whether the

Fig. 2 a, Viral DNA was prepared from plants that had been infected with SalIcut DNA of pCaMV10 (lane 1), pCaMV10-EOP1 (lane 3) and pCaMV10-EOP2 (lane 4). After cleavage by XhoI restriction endonuclease and labelling with  $[\alpha^{-3^2}P]dATP$  by polymerase I (Boehringer), the DNA was electrophoresed on a 10% polyacrylamide gel. Plasmid DNA pCaMV10-EOP1 (lane pCaMV10-EOP2 (lane 5) was treated in the same way. The 64-bp operator frag-ment of -EOP1 can be distinguished from 65-bp fragment of -EOP2, as indicated at the right-hand side. b, EcoRI restriction pattern of viral DNA derived from eight independent local lesion isolates of CaMV-1841-EOP1 and EOP2 that had been transferred to a set of new plants. Lanes 1, 6 and 11 contain plasmid from pCaMV10-EOP1, pCaMV10-EOP2 pCaMV10. and respectively. Lanes 2-5 contain DNA from four different isolates of CaMV1841-EOP1; lanes 7-10, DNA of four independent isolates of CaMV1841-EOP2. The EcoRI fragment that has been modified by the insertions is indicated by \*. The size of the fragments is given in base pairs. The introduction of the lac operator insert into the 2,006-bp EcoRI fragment also introduces an additional EcoRI restriction site and gives rise to two new fragments, the sizes of which (1,292 and 778 bp as opposed to 1,240 and 831 bp)



vary depending on the orientation of the insert. X indicates a submolar fragment present in all *EcoRI* digests of CaMV DNA<sup>7</sup>. The 4,524- and 3,009-bp fragments are 'hybrid' fragments containing CaMV and pBR322 DNA, their CaMV moiety is part of the 3,172- and 2,334-bp fragments of the viral DNA.

transcription patterns of CaMV 1841-EOP1 or 1841-EOP2 differ from that of CaMV 1841 has not been determined.

A packaging limitation of CaMV particles for additional DNA seems to restrict larger inserts—an additional 531 bp could not be successfully propagated in similar experiments. Thus there seems to be an upper limit for additional DNA of 256–531 bp. Recombinant molecules having large insertions in the CaMV genome may replicate, as a whole, only in those cells which become initially infected after inoculation. Perhaps encapsidation occurs only after sufficiently small deletions have arisen and may be a prerequisite for cell-to-cell movement and

COTAG COTAG



Fig. 3 Sequence comparison of the lac operator insert before and after passage through the plants. The respective HindIII restriction fragments (see Fig. 1) were subcloned in pBR322 (ref. 19). DNA of the hybrid plasmids was cleaved by EcoRI, labelled either with  $[\gamma^{-32}P]ATP$  (Amersham) and T4 polynucleotide kinase (Boehringer) or by Pol I Klenow fragment plus [α-32P]dATP to label the opposite strand. After cleavage with HindIII and preparation of the fragments on 6% polyacrylamide gels, the DNA sequence of the inserts was determined as described elsewhere 15. The figure shows an 8% sequencing gel of CaMV-EOP1 DNA (after passage) labelled with  $[\alpha^{-32}P]$ dATP (a)and pCaMV10-EOP1 DNA before passage through the plants (b). The readable sequence starts with 3' AACAAT 5' in the middle of the lac operator (see Fig. 1, upper strand).

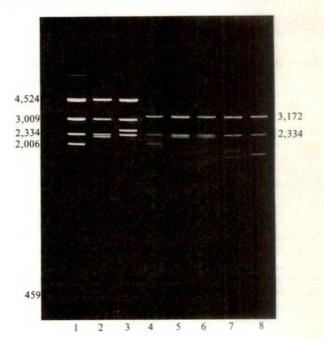


Fig. 4 EcoRI restriction pattern of Λ DNA insertions in CaMV1841. Lane 1, EcoRI-cleaved plasmid DNA of pCaMV10; lanes 2 and 3, pCaMV10 containing a 256-and a 531-bp insert of Λ DNA, respectively. The insertions at the XhoI site cause differences in mobility of the former 2,006-bp fragment. Lanes 4-8 contain viral DNA isolated from individual plants that showed symptoms after inoculation with the three plasmid DNAs cut by SalI. Lane 4 shows the wild-type CaMV1841 EcoRI restriction pattern. The mobility of the EcoRI fragment that contains the 256-bp insert seems identical to that of the parent plasmid (lanes 5 and 6). In contrast, the 531-bp insert produced three different deletions, each of which rendered the fragment carrying the insert smaller than the wild-type 2,006-bp fragment (lanes 7 and 8). Only the largest of these new fragments (lane 7) still contains sequences that hybridize to λ DNA (data not shown).

systemic development in the plant. Further experiments with a 363-bp insertion at the XhoI site of pCaMV10 support this assumption as there is an 'eclipse period' of >2 months before the plants start to develop symptoms of virus infection. Analysis of the viral DNA reveals that deletions occur which include part of the DNA inserted and part of reading frame II on the CaMV chromosome (B.G. et al., in preparation).

As insertion of foreign DNA in the XhoI site interferes with expression of the assumed reading frame II<sup>3,4</sup>, its product might be non-essential. Alternatively, this region may not code for any protein at all. The finding that the deletion in CM4-184 eliminates almost the entire coding region II agrees with these results12.

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Insertion of bacterial DNA has been useful in probing the genome of CaMV. We are now investigating whether there are other regions suitable for insertions and whether inserts of different kinds can be used to express new proteins in plants using CaMV as vector.

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#### Role of the *nifA* gene product in the regulation of nif expression in Klebsiella pneumoniae

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The nitrogen fixation (nif) gene cluster of Klebsiella pneumoniae is linked to his, consists of at least 17 genes arranged in seven or eight operons1-4 (Fig. 1), and its expression is inhibited by oxygen (O2) and fixed nitrogen (N) including ammonia, nitrate and amino acids (see ref. 5 for review). Previous studies have suggested that the nifA and nifL gene products respectively activate 6-8 and repress 9,10 nif expression. There has also been evidence, from mutants that express nif constitutively in the presence of fixed N, that regulation by fixed N involves the gln (glutamine synthetase) genes<sup>11,12</sup>, and that their products act at the promoter of the nifLA operon<sup>13,14</sup>. We have recently shown by direct measurement of transcription in vivo that the nifA and nifL gene products are, respectively, an activator and repressor of nif transcription initiation from all nif promoters except that of the nifLA operon15. We now report experiments aimed at clarifying the role of the nifA product and the interaction of the nifLA operon and gln genes in controlling nif expression. Having constructed plasmids that constitutively express the nifA gene and investigated nif expression in a variety of K. pneumoniae strains transformed by such plasmids, we show that, in the absence of nifL protein, nifA expression is sufficient to activate nif expression, even in the presence of fixed N and O2. gln-mediated regulation of nif by fixed N exerts its effect by controlling nifLA expression. We have also confirmed that the nifA protein is temperature sensitive, thus explaining the absence of nif expression at 37 ° C. Our results provide a strategy by which the constitutive expression of nif in the presence of fixed N and O2 is stabilized and suggest a model for nif transcriptional regulation.

To investigate the regulation of nif expression by fixed N and O<sub>2</sub> and to clarify the role of the nifA product, we have used a variety of K. pneumoniae strains transformed with plasmids permitting constitutive expression of the nifA gene. The gene was cloned as a SalI fragment (which also contained part of the 3' end of the nifL gene) in the multicopy vectors pACYC184 and pACYC177 (ref. 16). The fragment was inserted into the SalI

site of pACYC184 in both orientations, giving pMC71A and pMC71B; in pMC71A nifA was expressed from the promoter of the tetracycline resistance gene (Tc<sup>r</sup>). Insertion into the XhoI site of pACYC177 in both orientations gave pMC73A and pMC73B; expression of nifA in pMC73A was from the promoter of the kanamycin resistance gene (Km<sup>r</sup>).

Table 1 Nitrogenase activities of K. pneumoniae strains with and without nifA plasmids

Strain	Nitrogenase activity as % of that in derepressed UNF928 -NH <sup>+</sup> +NH <sup>+</sup>		
UNF928 (nif wild-type strain)	100	0.005	
UNF714 (nifA)	0	0	
714 (pMC71A)	97	15	
714 (pMC71B)	7	0.1	
714 (pMC73A)	230	27	
714 (pMC73B)	0.1	0.1	
UNF1780 (gln A100 → Nif <sup>-</sup> )	0.05	0.075	
1780 (pMC71A)	328	88	
1780 (pMC71B)	257	1	
1780 (pMC73A)	174	80	
1780 (pMC73B)	0.005	0.005	
UNF1827 $(gln A201 \rightarrow Nif^-)$	6	0.005	
1827 (pMC71A)	276	86	
1827 (pMC71B)	122	0.07	
1827 (pMC73A)	279	70	
1827 (pMC73B)	0.2	0	
UNF1829 $(glnG36 \rightarrow Nif^-)$	0.1	0	
1829 (pMC71A)	284	22	
1829 (pMC71B)	226	0.03	
1829 (pMC73A)	125	22	
1829 (pMC73B)	0.06	0.07	

Overnight cultures (4 ml) were grown anaerobically in bijoux bottles in NFDM<sup>22</sup> containing 25 μg ml<sup>-1</sup> histidine and 100 μg ml<sup>-1</sup> serine (UNF928, UNF714 strains) or 100 μg ml<sup>-1</sup> glutamine (UNF1780, UNF1827, UNF1829 strains) (-NH<sub>4</sub><sup>+</sup>). Serine was omitted in NH<sub>4</sub><sup>+</sup>grown cultures (+NH<sub>4</sub>) which contained 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Strains carrying pMC71A and B and pMC73A and B were selected with 25 µg ml<sup>-1</sup> chloramphenicol and 200 µg ml<sup>-1</sup> carbenicillin, respectively. Nitrogenase activity was measured by acetylene reduction as described in ref. 22. Relevant genotypes are: K. pneumoniae strains, UNF928 (hisD hsdR recA), UNF714 (hisD nifA hsdR recA), UNF1780 (hisD glnA100 hsdR recA), UNF1827 (glnA201hsdRrecA) and UNF1829 (glnG36hisD,hsdRrecA). UNF1780, UNF1827 UNF1829 do not express nifA or nifL, even in the absence of NH<sub>4</sub>, because they lack the necessary gln-mediated positive control.

**Table 2**  $\beta$ -galactosidase expression from the *nifH* promoter in strains with and without *nifA* plasmids

S	train	β- -N	galactos H <sup>+</sup>	idase uni +N	
1. UNF921			3		3
	(pMC71A)	1,9		2,2	-
	(pMC71B)	7	16	1	25
4. UNF931	(pJB31)	2,2	200		2
5. MC1061	(pJB31)		3		2
6. MC1061	(pJB31) (pMC71A)	6,0	)27	4,2	46
		-N	$H_4^+$	+N	$H_4^+$
		+O <sub>2</sub>	$\overline{-O_2}$	+O <sub>2</sub>	$-O_2$
7. UNF921		3	6	2	3
8. UNF921	(pMC71A)	2,250	2,160	1,570	3,000
9. UNF921	(pRD1)	22	2,200	. 8	6

Overnight cultures (4 ml) of strains 1-6 were grown anaerobically in bijoux bottles in NFDM<sup>22</sup> containing 25  $\mu$ g ml<sup>-1</sup> histidine except for UNF921 (pRD1), 100  $\mu$ g ml<sup>-1</sup> serine (-NH<sub>4</sub>) or 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> instead of serine (+NH<sub>4</sub>). Overnight cultures (10 ml) of strains 7-9 were grown in the same two media under a constant stream of air (+O<sub>2</sub>) or N<sub>2</sub>(-O<sub>2</sub>) in 100-ml conical flasks which were vigorously shaken. All cultures were grown at 28 °C.  $\beta$ -galactosidase activities were measured as described in ref. 8. Relevant genotypes are: *K. pneumoniae* strains, UNF921 ( $lazZ:nifH \nabla (his-nifH)recA hsdR$ ), UNF931 (hisD lac recA hsdR) and *E. coli*, MC1061 ( $hsdR \triangle (lacIPOZY)X74$ ). Plasmid pMC71A and B carry Cm<sup>7</sup> and nifA genes, pJB31 carries Ap<sup>7</sup> and lacZ:nifH genes and pRD1 is a conjugative plasmid carrying Ap<sup>7</sup> Tc<sup>7</sup> Km<sup>7</sup> his and nif genes. Details of pJB31 construction will be published elsewhere.

Expression of *nifA* from these plasmids was checked in a NifA<sup>-</sup> strain UNF714. The results in Table 1 show that pMC71A and pMC73A both complemented the *nifA* mutation in UNF714. The low level of activity obtained with pMC71B was probably due to expression of *nifA* from an unidentified promoter on pACYC184, because a significant level was not obtained with pMC73B, indicating that no transcription initiation occurs between *nifL* and *nifA*.

Table 1 lists the nitrogenase activities of several different K. pneumoniae strains with and without the nifA plasmids and in the presence and absence of NH<sub>4</sub>. Nitrogenase activity in the nif wild-type strain UNF928 is repressed in the presence of 10 mM NH<sub>4</sub>. However, strain UNF714, when restored to Nif<sup>+</sup> by pMC71A or pMC73A, shows 15-30% constitutive nitrogenase activity (activity in the presence of NH<sub>4</sub> as a percentage of that in the absence of NH<sub>4</sub>). Constitutive nif expression in the presence of NH<sub>4</sub> was also examined by measuring β-galactosidase activity in a strain (UNF921) with a nifH::lacZ fusion (Table 2). In this strain the *lacZ* gene is fused to the *nifH* promoter and *his-nifH* is deleted (M. Merrick, unpublished). Strain UNF921 carrying pMC71A shows 100% constitutive  $\beta$ -galactosidase expression from the *nifH* promoter. The difference in degree of constitutive expression at the transcriptional ( $\beta$ -galactosidase activity) and nitrogenase activity levels may be due to a physiological effect on nitrogenase activity in an NH<sub>4</sub><sup>+</sup>-grown culture. The effect of O<sub>2</sub> on nif expression could be examined in UNF921 (pMC71A); nitrogenase activity could not be used because the enzyme is inactivated by  $O_2$ . The results (Table 2) show that  $\beta$ -galactosidase expression was 70-100% constitutive in UNF921 (pMC71A) grown in the presence of O<sub>2</sub> with and without NH<sub>4</sub>.

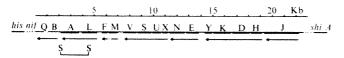


Fig. 1 Map of K. pneumoniae nif genes<sup>1-4</sup>. The arrows indicate nif operons and their directions of transcription. The SalI restriction fragment used in the construction of nifA clones is denoted by S-S. Kb, kilobases.

Thus these results show that nifA protein is active in the presence of fixed N and  $O_2$ , and that in the absence of nifL protein only the nifLA operon is regulated by fixed N or  $O_2$ .

To examine the possibility that the nifA protein is the only nif gene product required for transcriptional activation of the nifH promoter, we constructed a small, multicopy plasmid, pJB31, carrying a translational nifH::lacZ fusion. nifH and lacZ were fused by Bal31 digestion from codons 147 and 8 of the respective genes followed by blunt-end ligation and selection of clones which had  $\beta$ -galactosidase activity. The results in Table 2 show that lac was not expressed from pJB31 in an Escherichia coli strain, MC1061, while in the Nif+ strain UNF931 lac expression from pJB31 was under normal nif regulation. When pMC71A was also present in MC1061, lac was expressed at a high level from pJB31 even in the presence of NH4. Thus, the only nif gene product required for activation of the nifH promoter is that of nifA. SDS-polyacrylamide gel electrophoresis of pulselabelled extracts from UNF921 (in which his-nifH is deleted) and UNF921 (pMC71A) also indicated that the nifA protein was the only nif gene product required for expression from the nifJ promoter.

The constitutive expression of nif in UNF714 carrying pMC71A or pMC73A suggested that gln-mediated regulation of nif expression acted only at the promoter of the nifLA operon. To test this hypothesis we investigated the effect of the nifA plasmids on nif expression in three gln mutants, which normally do not express nif, presumably because a gln protein regulates transcription of the nifLA operon. The results (Table 1) show that the expression of nif was independent of gln when plasmids that constitutively expressed nifA were present in these mutants, indicating that only the nifLA promoter was directly regulated by the gln system.

The plasmid pMC71B restored higher nitrogenase activity to the Gln strains than to UNF714 (Table 1), perhaps due to the absence of nifL expression in the gln mutants. We have found that the nifL protein repressed nif transcription<sup>15</sup> and it is possible that in its absence less nifA protein is required to give a high level of nif expression. A result consistent with this observation is that a nifL mutant derepressed at a faster rate and gave

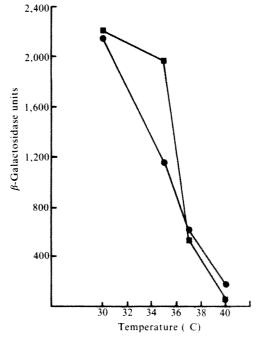


Fig. 2 Effect of temperature on *nif* expression in UNF921 (pMC71A) ( $\blacksquare$ ) and UNF921 (pRD1) ( $\blacksquare$ ). Cultures (10 ml) in 25-ml conical flasks were grown anaerobically in NFDM containing serine (100  $\mu$ g ml<sup>-1</sup>)<sup>22</sup>. UNF921 (pMC71A) cultures also contained histidine (25  $\mu$ g ml<sup>-1</sup>) and chloramphenicol (25  $\mu$ g ml<sup>-1</sup>). After overnight growth at four different temperatures  $\beta$ -galactosidase activities were measured as previously described<sup>8</sup>.

a higher level of nitrogenase activity than the wild-type (ref. 17 and M.C.C., S. Hill, E. Kavanagh and F.C.C., in preparation). The repression of nif by NH<sub>4</sub> in the gln mutants carrying pMC71B is probably due to a blocking of nifA expression by vigorous transcription from the Tcr promoter in the opposite direction. We have found that transcription from the Tc promoter blocked transcription from the opposite direction in a similar promoter arrangement<sup>15</sup>.

Nitrogenase from K. pneumoniae is active at 37 °C but nif is not expressed at this temperature 18. There is evidence that this temperature sensitivity is mediated by the nifL and/or A gene products<sup>17,19</sup>. We therefore examined the effect of temperature on the activity of nifA protein by comparing  $\beta$ -galactosidase expression in the nifH::lacZ fusion strains UNF921 (pMC71A) and UNF921 (pRD1) at different temperatures. Strains carrying pACYC184 grow in the presence of Tc at the temperatures used and this shows that transcription initiation from the Tc promoter on pMC71A is not temperature sensitive. Figure 2 shows that sβ-galactosidase activity decreased in both strains with an increase in temperature. As nifA protein is constitutively synthesized from the Tcr promoter in UNF921 (pMC71A) and no nifL protein is present, this result shows that the nifA protein is temperature sensitive. A similar result was obtained with a plasmid on which nifA alone was expressed from the nifLA promoter, which is known not to be temperature sensitive  $^{17,19}$ . The *nifA* protein has some activity at 37 °C and the observation that nifL mutants express nif at 37°C17 is probably due to a lower requirement for nifA protein in the absence of a functional *nifL* protein (see above).

Our results show that *nif* expression can be switched on

constitutively by inactivating nifL and fusing nifA to a constitutive promoter. These and other recent results provide strong evidence for the following model of nif transcriptional regulation. In nif derepressing conditions a gln protein activates transcription of the nifLA operon. The nifA protein then activates transcription of the other nif operons while the nifL protein is maintained in an inactive form by interaction with a regulatory metabolite. A probable candidate for this role is the purine nucleotide ppGpp which has an important regulatory role in conditions of N starvation<sup>20,21</sup>. When fixed N or O<sub>2</sub> is added to a derepressed culture the level of the regulatory metabolite is decreased and the nifL protein becomes a repressor of transcription at the promoters of all the nif operons except that of nifLA. A rapid decrease in the level of ppGpp in these conditions would lead to the transition of RNA polymerase to a different structural form<sup>21</sup> which would probably be less efficient at initiating transcription from *nif* promoters. Therefore, the repressor activity of nifL protein and the altered RNA polymerase would both contribute to a rapid nif switch-off on addition of fixed N or O<sub>2</sub>. In the presence of fixed N a gln protein represses transcription from the nifLA promoter, thus concomitantly removing the nif repressor and activator. Repression of nif in cultures grown in N-rich media would therefore be maintained solely by the absence of the nifA protein.

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#### cDNA sequences of human glucose 6-phosphate dehydrogenase cloned in pBR322

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Glucose 6-phosphate dehydrogenase (G6PD) catalyses the first reaction of the so-called oxidative pathway of glucose metabolism1. In many cells, in addition to providing pentose sugars required for nucleic acid synthesis, G6PD has a major role in providing an adequate supply of reducing power in the form of NADPH (ref. 2). The enzyme has been purified and characterized from many sources'. G6PD from mammalian cells consists of a homodimer or a homotetramer, with a subunit molecular weight of ~58,000 (ref. 4), and its structural gene is located on the X chromosome<sup>5</sup>. Genetic variation of G6PD is extensive in humans. Many variants associated with relative deficiency of enzyme activity ('G6PD(-)') are polymorphic in various populations and have probably been selected for by Plasmodium falciparum malaria<sup>6</sup>. A number of G6PD(-) variants can cause either acute or chronic haemolytic anaemia and therefore have considerable clinical importance<sup>7</sup>. We considered that a knowledge of the G6PD gene would be of interest because it may be representative of a large number of genes and could help to elucidate in detail the basis for various forms of G6PD deficiency. Here we report how G6PD cDNA sequences have been cloned starting from human mRNA in which G6PD mRNA is present at an abundance of less than  $10^{-4}$ 

Human diploid fibroblasts were grown from a primary skin explant by standard techniques<sup>8</sup>. Approximately 10<sup>9</sup> cells were used to prepare total RNA by the guanidine HCl method9. Enrichment for G6PD-specific mRNA was obtained by successive steps, consisting of oligo(dT)-cellulose chromatography and two successive sucrose gradient centrifugation runs, as will be described in detail elsewhere (D.T., M.G.P., G. Battistuzzi and L.L., in preparation). The purification was followed by a specific assay based on cell-free translation (see Fig. 1). From the enriched mRNA (Fig. 1, tracks 4, 7), cDNA was prepared using oligo(dT)-primed reverse transcriptase according to Sobel et al.  $^{10}$ , except that the concentration of oligo(dT) was 5 µg ml $^{-1}$ and that of each deoxynucleoside triphosphate was 1 mM. From this cDNA, double-stranded DNA was obtained using Escherichia coli DNA polymerase<sup>11</sup>, and after C-tailing<sup>12</sup> it was annealed to G-tailed plasmid pBR322 (ref. 13) and used to transform E. coli HB101 (ref. 14). After plating on tetracyclineagar plates, 4,750 colonies were observed, of which 3,470 were found to be ampicillin sensitive, indicating that they contained a plasmid with a double-stranded DNA insert. From an estimate of the abundance of G6PD mRNA in the reverse-transcribed preparation, we expected that 5-10 should contain G6PD sequences

The 3,470 colonies were screened by two approaches. First, RNA from cultured HeLa cells was purified as outlined in Fig. 1

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# **BOOK REVIEWS**

# Darwin through a purple haze

A.J. Cain

THERE are already far too many books about Darwin, and most of them tell us more about their authors than their subject-matter. It would be an injustice to leave Mr Brent's book in that category without considerable qualification. It will certainly be widely read, and for many people it may be their first introduction to Charles Darwin's mental, moral and hormonal development. Mr Brent is a wellpractised writer — he has already produced three novels and, according to the dust jacket, "ten pseudonymous thrillers, a study of Hindu gurus, two narrative histories, and several biographies". When he gets down to straightforward narrative and to psychological surmise, he is in his element; and he has certainly worked over a great deal of unpublished material relating to Charles's relations with his father, his sisters and other relatives, his early flames, his wife and his numerous friends. He has also profited, as he makes clear, from a number of other studies of Darwin and Darwinism: to that extent, the book is one of the best and most up-to-date accounts of Charles Darwin as a man that one can find.

Unfortunately, it is far from satisfactory in a most predictable way; the science in it is so light-weight that yet again we have Hamlet without the Prince, a sort of Himmelfarb rediviva. When will people of only literary training (and I include philosophers and some historians) realize that to write the history of a scientist they must intuit not only into his complexes and sex life but into his science as well?

The book is obviously aimed at the general market to be created by the centenary of Charles Darwin's death, already partly excited by the anti-Darwinian rubbish recently appearing in the popular press and on television and radio. To ensure a wide acceptance, the author has decorated it freely with purple passages which do not accord well with the generally excellent style in the narrative parts. Indeed, the first page after the Introduction, beginning "The boy is sturdy, high-complexioned, with fine, chestnut-coloured hair now in some disarray" and containing the remarkable sentence "Briefly, in delighted alarm, pigeons flick to and fro against the bulbous clouds" may even put off some who would find better things later.

Much of this could be disregarded, were not the same inflation constantly used on Charles Darwin himself. His attitude towards marriage was somewhat "monstrous" (p.249), his approach to his main task was "almost inhumanly thorough" (p.281), he was capable of "an

Charles Darwin: A Man of Enlarged Curiosity. By Peter Brent. Pp.536. ISBN UK 0-434-08595-2; ISBN US 0-16-014880-2. (Heinemann, London/Harper & Row, New York: 1981.) £12.50, \$20.75.

almost frightening objectivity" (p.320); many such expressions combine to build a picture of almost a maniac. They are reinforced by numerous suggestions of duplicity (p.187), hypocrisy (p.475), eagerness to duck criticism (p.229) and so on, which make the book a God-sent mine of reference for any fundamentalist wanting to denigrate Darwin. It is true that Mr Brent, having made these remarks often qualifies them; but as Paul Hazard reminds us, so-called refutations of Spinoza served to spread his ideas, and many of Mr Brent's self-rejected suggestions have now got into print. In much of what he judges so harshly, I see little more than very human vacillations, and not infrequently early Victorian modes of expression which he has taken rather too

Mr Brent has done his best with Darwin's sex-life, in spite of a plentiful lack of material; indeed, at one point he speaks of the prurience of a biographer. His suggestions about Charles's relations with his sisters, and their over-mothering him are excellent, as is his perceptive account of his relations with his father. But when (p.263) he sees Emma as offering Charles another aspect of the same archetype, Lady of the Manor and Peasant" he himself comments that "perhaps it gives it [this strand in their relationship] a false importance even to select it for comment" and goes on immediately to emphasize it. And he just cannot resist a remark of one of Charles's female friends (p.61) regretting she was not there to meet him and "to make a beast of myself in the strawberry beds". Mr Brent gets quite excited about those strawberry beds, and his comment "One sees them [Fanny and Charles] at their languid orgy, half children, half adults" etc. etc. is worthy of Cold Comfort Farm; the beds recur again on p.140 ("the sprawling hours among the strawberry beds") and even again on p.501 ("the young Charles delirious with summer among the strawberry beds" etc.).

A great deal of what Mr Brent writes is well-written, sensitive, perceptive; it is a pity he should spoil the book, both in this way, and by setting up straw men to demolish, and by suggesting mysteries where they hardly exist in order to overemphasize Charles's achievements. To

refer to Charles as "the hearty outdoor dullard of legend" makes one curious as to what reputable biographer ever suggested he was a dullard. To refer to Cuvier as enigmatic, or Lamarck as brilliant but enigmatic hardly does justice to their biographers, let alone the men themselves.

Equally, in the opposite direction, Charles Darwin did so much so well that he does not need to have ascribed to him ideas which he took from others. His vision of the Unity of Nature (p.289) was a commonplace to his grandfather, as were the ideas of trees as compound animals (p.317), sensitive plants as furthering the analogy between animals and plants (p.308) and the female bosom as an object of special interest (p.319), even, to Erasmus Darwin and others, the origin of the curve of beauty.

Except in dealing with the Galapagos Islands and Darwin's finches, which have been too well written-up to allow serious mistakes, Mr Brent hardly touches without error the actual science which Darwin so avidly investigated and extended. Amphioxus is tropical only if Plymouth is too. Megatherium appears on p.156 as "an enormous forerunner of the giant sloth" and on p.198 as "the prehistoric armadillo" - in fact it was one of the giant sloths and not a precursor of anything. The "genus Brachipoda" (p.403) is a hopeless misnomer, as is the "species Canidae" (p.472), and the cirripedes had not been molluscs since Lamarck (1818). Mr Brent has no patience with the mere details of natural history, like other authors I have reviewed recently, yet to Darwin they were the absolute basis of his science. It is not surprising that Mr Brent cannot really understand why Darwin laboured so long at his barnacles, and has to put it down to Darwin's character in the end.

It is clear from Mr Brent's references to sexual selection (p.480) that he has no more understood the present standing of that topic than Gertrude Himmelfarb understood the present standing of natural selection. To comment on sexual selection that the exercise of volition by a female

... muddled the simplicity of the original vision. It was an element which conflicted with the impersonal logic of a selection process that derived only from the interaction of organism and environment....

is to show that what fascinates Mr Brent is ideas only (not their relevance to the actual world which is what fascinates a scientist). For him, natural selection "has become a staple of the everyday rhetoric through which we now define ourselves" (p.509) and it is his lack of knowledge of actual

work on natural selection that enables him to repeat the old cliché. What he calls the underlying logic of evolutionary theory "seems to say no more than that survivors survive". This hoary fallacy has been refuted many times, but he does not say so.

Mr Brent must surely have a considerable readership; his abilities in his own line are outstanding. The present book should be asked for as "the latest Peter Brent", rather than as a successful study of Darwin. No scientist need bother with it.

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#### Rare air of motion

R. Hide

Dynamics of the Upper Atmosphere. By Susumo Kato. Pp.233. ISBN 90-277-1132-1. (Reidel: 1980.) Dfl. 57, \$29.95.

TECHNIQUES for investigating high-level atmospheric motions have advanced considerably over the past 20 years, with the introduction of rockets, satellites and high-powered radar on the observational side, and the advent of computers for the analysis of data and their theoretical interpretation in terms of basic physical processes.

Professor Sato's monograph — the first in a series, Developments in Earth and Planetary Sciences, stressing Japanese work but written in English - is a mathematically orientated introduction to the study of these motions and it reflects not only the author's main research interests but also (I imagine) his activities as a teacher of applied mathematics. The book deals in a straightforward and fairly standard way with the basic equations of dynamics, thermodynamics and electrodynamics applied to the atmosphere. These equations are used to describe the main properties of acoustic gravity waves and atmospheric tides and their manifestation in observations of the ionosphere. The presentation is strongly slanted towards those who are primarily interested in mathematical aspects of atmospheric wave propagation but many observational data are given in this profusely illustrated book, with its 106 diagrams and many tables.

Most atmospheric physicists would find something useful in the book, but it is possibly too narrow and, in parts, too detailed to be useful as an introductory text. The standard of English leaves much to be desired in places but this can hardly be blamed on the author, who should have been better served by his publisher.

## News from the petroleum geologists

J.R.V. Brooks

Petroleum Geology of the Continental Shelf of North-West Europe, 1981. Edited by L.V. Illing and G.D. Hobson. Pp.521. ISBN 0-85501-656-6. (Heyden & Son: 1981.) £47, \$113.

It is now some 16 years since the first discovery of offshore gas in UK waters, and 13 years since the first commercial discovery of oil. In the period from 1964 to the present nearly 1,800 wells have been drilled.

To those involved in exploration and in the production of oil and gas these have been, and still are, exciting times filled with geological surprises. But to many geologists — nearly a generation of them the scientific results of drilling have not been easily available, and it is increasingly apparent that universities schools - do not have the finances to purchase the data that now exist. Study of the geology of the UK and continental landmass of Western Europe gives only a partial picture of the total geology and geological history of the area. But when this is linked to what is now known about the North Sea Basins and other offshore areas, a more complete and meaningful picture emerges. From a teaching point of view, and to large numbers of geologists in non-oil areas, it is important that this new information becomes incorporated into the present state of knowledge.

Up-to-date data are hard to come by, but from time to time the industry holds a conference during which current views are aired and papers on exploration and proven fields published. The first conference on the petroleum geology of the continental shelf of North-West Europe was held in 1974, and the second in 1980 in London; it is the proceedings of the 1980 meeting which form the contents of the volume reviewed here.

The number (46) and range of papers presented is great; they are grouped into four main categories covering regional aspects of geology, technological developments, stratigraphy, and selected oil and gas fields. In some cases the opportunity has been taken to update material presented at the 1974 conference, but the majority of papers record more recent and current work, and present previously unpublished data.

Certain contributions were specially commissioned by the conference committee, notably those on temperature and thermal gradients in the North Sea, the regional seismic maps of the North Sea, and the subcrop map of the mid Mesozoic unconformity, the latter providing a particularly useful overview.

Clearly one cannot mention every single contribution and all of them are worthy of study, but there are a number of more significant papers that represent milestones

in exploration activity. Of these, the Brae field is as near as we have come to finding a stratigraphic accumulation of oil and its discovery has led to a search for further accumulations of this type. The discovery of the Frigg field is perhaps a unique example of a sedimentary unit which can be readily identified on a seismic section and might be described as a seismic/structural accumulation. In fact it is described as a preserved deep sea fan.

The largest discovery of oil onshore, made in 1973 at Wytch Farm, set off renewed interest in landward hydrocarbon exploration, which is currently being sustained. The results of this discovery have had considerable geological implications for exploration elsewhere in the UK, and the paper on Wytch Farm sets out the results and regional setting of the discovery.

Each of the papers in the volume represents recent results of exploration activity and might be considered as highlights of the book, published as they are for the first time. But the regional and field evaluations also serve to give the technical reader a wide overview of current hydrocarbon activity.

Such is the vast range and wealth of geology presented in this volume that no geologist, particularly those whose task it is to teach the subject, should be without a copy. Sadly, however, its cost may preclude the sort of distribution its contents deserve.

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# Greater clarity in lens biology

Christine Slingsby

Molecular and Cellular Biology of the Eye Lens. Edited by Hans Bloemendal. Pp.469. ISBN 0-471-05171-3. (Wiley: 1981.) £50.65, \$91.15.

PROGRESS in eye lens research, whilst resulting in a greater understanding of the organelle itself and its diseases, may also lead to discoveries in areas of more general interest. In his selection of ten contributions for this book, Professor Bloemendal has placed emphasis on the growing interest in cytoskeletal structures, as the role they play in controlling and maintaining cell shapes can be well observed in lens cells. To this end, the chapter by Lucio Benedetti et al. is

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outstanding as a comprehensive yet coherent review of the biochemical, structural and physiological aspects of lens membranes in association with cytoskeletal filaments. By treating this aspect of lens cellular biology in the context of what is known from other organelles, the authors make obvious the advantages of this system and indicate promising areas for further research. The way forward is further underlined by Ramaekers and Bloemendal when they argue the case of the influence of the cytoskeleton on lens cell differentiation, bringing to call many exquisite illustrations.

The book makes clear the many advantages in studying this simple one-type cellular organ and it is not surprising that many people have spent time in characterizing crystallins, the lens structural proteins. These turn out to be not at all simple, but thankfully one book can only produce one classification system; a consistent usage here has led to a reasonable degree of coherence among the different authors. This classification of crystallins, complex though it must be, is where much of lens molecular biology begins and ends. It is thus a pity that where further explorations of molecular structures have been attempted, such as those resulting in a proposed model for the quaternary structure of a-crystallin, they are barely mentioned. However, there is an excellent chapter by Wilfried de Jong in which genetic relationships among crystallins are inferred from recently obtained amino acid sequences. By surveying the variability of lens crystallins throughout the animal kingdom and unearthing some obscure information relating to the functions of these diverse lenses, de Jong has produced a valuable essay on lens evolution.

In many of the chapters considerable space is given to experimental procedures and in parts this becomes an intrusion, especially when only the contributors' data are covered. In other areas the findings of all investigators and all points of view are discussed but with no synthesis; hence large tracts are unreadable. Not so, however, is the chapter by John Harding on the question of cataract research, in which he challenges most of the conventional cataract hypotheses by discussing the assumptions and contradicting the experimental data on which they are based. As a consequence, he strikes out a large body of lens research, thus rendering the book considerably shorter than it otherwise could have been.

The heroine of this book is the lens itself: the molecular story of its refractile transparency is still untold. But by presenting the background to important questions, the book can be recommended to anyone who wishes to look for the answers.

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# Australia's biogeographical development

Peter D. Moore

Ecological Biogeography of Australia. Edited by Allen Keast. Three volume set, pp. 2,182. ISBN 90-6193-092-8. (Dr W. Junk: 1981.) Dfl. 950, \$495.

THIS is an impressive work by any standards, even those of the publishing house of Dr W. Junk, which is renowned for its quality of production of specialized texts. Now, three volumes replace the previous book in the series dealing with Australian biogeography, published in 1959. It says much for the development of detailed knowledge of the Australian biota, and for the conceptual advances within biogeography as a science, that it has been necessary to rewrite and expand the original text within this time span.

The first volume deals with the evolution of the physical environment and its biota, and with the plant life of Australia; the second is largely concerned with invertebrates, fresh water biology and poikilothermic vertebrates; and the third covers homeothermic vertebrates, including man.

The development of the Australian environment is one area where great advances in both theory and the accumulation of raw data have taken place in the past two decades. Much attention is therefore given in the early part of the first volume to the break-up of eastern Gondwanaland and also to climatic changes during the Tertiary. Another important advance in the past ten years has been the analysis of Quaternary deposits using pollen analysis, and an integration of these data is briefly presented by Kershaw.

An interesting chapter on fire is rather uncomfortably inserted at this point, presumably because of its relationship to floral and faunal evolution, but it would have been better placed after the vegetation descriptions which follow.

The phytosociological account of Australian vegetation by Specht has more of a taxonomic than an ecological ring to it, but has some very valuable and novel components, such as the representation of C3 plant species in the vegetation formations. Some distribution patterns of selected taxa are also discussed. Specht follows this chapter with an account of the ecophysiological aspects of the vegetation. Plant-climate interactions, particularly in relation to drought and temperature, dominate this chapter, but some attention is also given to soil nutrients and the role of fire.

There follows an unconformity in that the historical approach is reinstated for the detailed consideration of Australia's fossil flora and the development of phytogeographical regions. Again, one wonders whether a more natural sequence of topics could have been arranged. For example, Hélène Martin's detailed account of the Tertiary flora would have been better

placed adjacent to the Tertiary climate section and before that dealing with the Quaternary flora. The first volume is completed by a series of papers each dealing with a separate taxonomic group of plants, such as *Eucalyptus*, the grasses, the lichens and so on, or with particular environments, such as the high mountains or the deserts. These varied approaches provide a wealth of information on the Australian flora.

The arrangement of the second and third volumes is almost entirely taxonomic, though the emphasis is generally on the evolutionary development of the group being discussed. These specific accounts of groups are interspersed with more general overviews and reviews of such subjects as the biogeography of terrestrial invertebrates or of aquatic insects. The second volume concludes with fishes, amphibians and reptiles. The arrangement of material here, by taxonomy, leads to a certain amount of repetition between chapters, particularly those relating to habitat distribution and the history of land masses.

The final volume, concerning homeothermic vertebrates, naturally spends much time on the evolutionary development of Australia's mammals, with particular emphasis upon the relationship of marsupials to arid climates and upon the late arrival in Australia (5-4x 10° years ago) of rodents. Birds also occupy a position of prominence, and a paper by Kikkawa et al. on the biological history of the Cape York peninsula, where plant and animal interchange with New Guinea has been concentrated, is worthy of especial mention.

It is refreshing to see a work of this kind in which man can be treated not simply as an additional factor of the environment, but as an organism with a biogeographical story of his own. The prehistory, physiology and anthropology of Australia's aboriginal population gives ample opportunity for this kind of treatment. The relative importance of man's arrival, about 60,000 years ago, and climatic changes since that time upon Australia's fauna is difficult to unravel, but Norman Tindale presents a graphic summary of the evidence.

This superbly produced set of books can be criticized only in that it has attempted to cover so vast a mass of information that arrangement and editing has evidently been a problem. Systematic and subject indexes are included, the former comprehensive, but the latter rather sketchy. These volumes establish a data base and reference source upon which biogeographical research in Australia will be built for many decades to come.

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## Anamorph anthology

C.T. Ingold

Biology of Conidial Fungi, Vols 1 and 2. Edited by Garry T. Cole and Bryce Kendrick. Vol.1, pp.486, ISBN 0-12-179501-2; Vol.2, pp.660, ISBN 0-12-179502-0. (Academic: 1981.) Vol.1 \$49, £32.40; Vol.2 \$68.50, £45.40.

An alternative title for this work might have been All About Anamorphs, yet ten years ago this would have meant nothing to a mycologist. Now we accept a whole terminology of "morphs", much to the fore in Biology of Conidial Fungi. "Morph" is sometimes used in all its nakedness, but more usually as a termination: the teleomorph is associated with sex and meiosis; the anamorph with the asexual, conidial stage; and the holomorph is the whole fungus. The holomorph ideally is a teleomorph together with its anamorph, but it may consist of a teleomorph only, or even solely of an anamorph.

The present work deals with conidial fungi which are anamorphs of Ascomycetes or Basidiomycetes, together with forms in which the "perfect" stage is not yet known or has, perhaps, been lost in the course of evolution. The conidial types in the "lower" fungi, notably Mucorales, are not considered.

During the past decade or so Bryce Kendrick has devoted much of his abundant energy to promoting the study of conidial fungi, especially by his organization of the original Kananaskis workshop summarized in Taxonomy of Fungi Imperfecti (University of Toronto Press, 1971), and of the second in the same place devoted to the linkage of conidial anamorphs with their teleomorphs. This gave birth to the two volumes of The Whole Fungus (National Museums of Canada, 1979). Now, together with Garry Cole, a distinguished student of the fine structure of fungi, he has persuaded 35 authors from all over the world to cooperate in writing 31 articles on various aspects of conidial

The articles are grouped in seven sections: history; systematics; distribution and ecology; conidial fungi and man; ultrastructure, development, physiology and biochemistry; genetics; and techniques for investigation.

• The first section has but one article, written by Bryce Kendrick, who also provides the initial chapter of the section on

Macmillan Reference Books, London (in North America, Facts on File) have recently published *The R.A.E. Table of Earth Satellites, 1957 -1980,* compiled by D.G. King-Hele *et al.* at the Royal Aircraft Establishment. The *Table* contains a chronological list of 2,145 launches and mention of some 12,000 satellites and associated fragments, details being given as appropriate. Price is £30.

systematics. One might, perhaps, expect these contributions to be a trifle dull; but not so. All that he writes throbs with enthusiasm and makes them a joy to read. The systematics section is one of the longest (260 pages), involving discussion of Coelomycetes, conidial yeasts, dimorphism, pleomorphism, conidial fungi growing on other fungi and lichens, and relations between conidial anamorphs and their teleomorphs. Nag Raj, writing about Coelomycetes, hands down ten exacting commandments for those rash enough to tackle these difficult organisms. Hawksworth contributes over 60 pages on fungicolous fungi. Although this is somewhat of an inflated list, it directs attention to these organisms which are much in need of detailed study.

In the ecological section, the chapters by Webster and Descals on fungi from freshwater habitats, and by Lacey on aerobiology, are particularly noteworthy and call for special commendation.

There is wide coverage of the economic importance of conidial fungi in connection with parasitism of crop plants, dermatophytes attacking man, mycotoxins, food spoilage and food technology, and the possibility of using certain species in the biological control of various parasites and pests. Although the fungi that prey on nematodes may not have much potential in such control, Barron's chapter on this subject is delightful.

Ultrastructure has a good innings with an illuminating chapter by Garry Cole. Nuclear behaviour is treated by Robinow in his usual masterly way. There is an interesting article by Lemke on fungal viruses and a splendid contribution by Aronson on hyphal walls.

The only chapter in the genetics section is by Hastie. This is outstanding, a real gift to mycologists at all levels.

The whole work is a major contribution to mycology and, although inevitably lacking some coherence, should find a place in all institutions where fungi are studied.

C.T. Ingold is an Emeritus Professor in the University of London, and a former President of the British Mycological Society.

# Beyond mechanics in human reproduction

R.V.Short

Conception in the Human Female. By Robert G. Edwards. Pp.1,087. ISBN 0-12-232450-1. (Academic: 1980.) £48.50, \$116.50.

DR Edwards is to be congratulated on having produced what may well turn out to be the most influential text in the field of obstetrics and gynaecology published this century. Hitherto, these specialities have been surgically orientated, and principally concerned with the mechanical difficulties of delivering babies, repairing prolapses and treating gynaecological cancers. This they have performed well, as attested in part by the maternal and perinatal mortality statistics.

Nonetheless the time has come for the profession to rise to a new challenge. Advances in our understanding of endocrinology, infertility, contraception and gamete biology have overtaken the surgical aspects of the subject, and one hopes this textbook will serve as a basis for the training of the obstetrician/gynaecologist of the future. He or she now needs to know more about endocrinology than those in general medicine, more about male reproduction than the urologist, and needs to develop a new understanding of genetics, reproductive biology, embryology, immunology and human sexuality. It is no accident that this excellent book was written by somebody who is not medically qualified; it is time for obstetricians and gynaecologists to rise to the challenge and develop their scientific understanding in parallel with their manual skills.

Dr Edwards has obviously chosen to discuss those topics that have been of greatest concern to him in the past 18 years in Cambridge, where in collaboration with Patrick Steptoe he pioneered the technique of human in vitro fertilization against open opposition from Nobel Laureates and masterly inactivity on the part of the Research Councils. Each chapter in the book is extensively referenced, and absolutely up-to-date at the time of going to press in early 1980. It is also particularly well illustrated, using key graphs, tables, diagrams and photographs taken from the work of others, duly acknowledged. The fact that Dr Edwards has been a stimulating teacher of undergraduates in Cambridge is reflected in the text — he knows what to discuss, and how to present it in the most exciting and informative

It will be disappointing if the word "Human" in the title means that this book will be restricted to medical libraries, and the bookshelves of consultants who are able to afford it. It deserves a far wider readership. Although principally concerned with human beings, Edwards takes a broad approach, and is not inhibited from discussing the wider comparative aspects of the subject. The book is destined to become a "must" for anybody working in the general field of reproductive biology — a treasured possession. For it really is excellent.

R.V. Short is Director of the MRC Unit of Reproductive Biology, Edinburgh.

# OKS REC

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## NEW ON THE MARKET

B.O.D. system. Wheaton Instruments have introduced the 60-second B.O.D. system for determining dissolved oxygen concentrations in liquid waste samples. This system measures samples precisely in <1 min to accuracies comparable to that of the Winkler method or others using electrodes. This accuracy is maintained even if the membrane becomes fouled, and no membrane replacement is required. There is no contamination or consumption of electrodes, and no oxygen is depleted from the sample. Air calibration is accomplished without having to dry the probe. The system is supplied complete with probe, cable, a.c. line cord, and complete operating instructions, including a temperature/altitude calibration table.

Circle No. 100 on Reader Enquiry Card.



Automatic curve plotting. Designed for use with the Dianagraph chart recorder/data logger, a calculation and control package is now available from Bailey Instruments. This unit enables the Dianagraph to process data in real time. For example, when channels 1 and 2 record voltage and current respectively, they can be multiplied to plot watts on channel 3. By integrating, a printout of W h can be produced automatically on channel 4. Data of wide dynamic range can be compressed using the logarithm function; rate of change of any parameter can be plotted separately using the differentiating mode. The basic Dianagraph produces analog recorder traces and digital printouts on the same paper. Using this feature, the calculation package in the integrating mode automatically measures the area beneath any curve, and prints it out in W h or any other units.

Circle No. 101 on Reader Enquiry Card.

Stopped-flow/T-jump spectrometers. The HI-TECH SF-30 series, with the accuracy and high data processing speed of an online minicomputer, incorporates a precisely controlled temperature range of -100 to +130 (±0.1)°C. A new modular accessory, the SFL-37 cryobiochemical module, allows controlled low temperature stopped-flow/T-jump work on cryobiochemical samples prepared quickly and easily, avoiding the usual curdling or separating problems.

Circle No. 102 on Reader Enquiry Card.

Gas chromatography. The Accuspec compact GC has an externally small format, but gives high performance usually associated only with larger units. The spacious, dual column oven with forced, ducted air circulation and instrument layout allow easy, effective coupling to auxiliary instruments.

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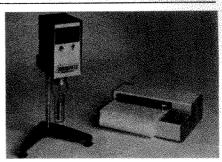
Monoclonal antibody screening. Four new monoclonal antibody screening systems, each using the ELISA assay, have been introduced by New England Nuclear. These systems provide all the components needed to screen for murine immunoglobulins, including enzyme-conjugated second antibody, colour development agent, and dilution, washing and substrate buffers. The reagents are pre-tested and optimized to prevent variables from causing unreliable results. Rapid and dramatic colour develops in the presence of positive clones. Two systems, sheep F(ab'), anti-mouse immunoglobulin and goat anti-mouse immunoglobulin, are provided with conjugated horseradish peroxidase. Circle No. 104 on Reader Enquiry Card.

Urinary amino acid controls. Human urine controls containing known levels of amino acids have been developed by Quantimetrix Medical Industries. These lyophilized controls may be reconstituted by the addition of distilled water. Disease states in which the urinary amino acid level may be increased include viral hepatitis, diabetes mellitus, hyperparathyroidism, vitamin deficiency, phenylketonuria and lead poisoning. The urinary controls also provide a reference for electrophoresis, thin-layer or column chromatography, spot testing or other amino acid analyses.

Circle No. 105 on Reader Enquiry Card.

Microcomputer interfaces. Anaspec have produced a new range of microcomputer interfaces constructed on a modular basis. These interfaces can be used with many different microcomputers, for example, the Commodore PET, Hewlett-Packard range, Apple and Superbrain. The input/output modules include analog to digital and digital to analog converters, relay closures and stepper motor controllers.

Circle No. 106 on Reader Enquiry Card.



Viscometer. The new digital viscometer from Brookfield Engineering allows continuous sensing for evaluation of time-dependent materials. The new viscometer is compatible with all Brookfield accessories and can be applied to all existing Brookfield viscosity applications. It features a large easy-to-read LED display and a 0-10-mV output signal. Optional equipment is an 8-inch chart recorder which provides a permanent record of rheological characteristics.

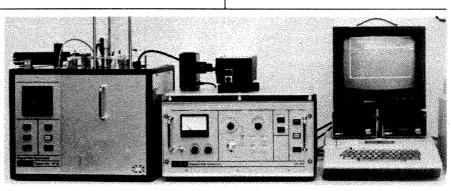
Circle No. 107 on Reader Enquiry Card.

Pyranometer solarimeter. The new model CM 11 pyranometer from F.T. Scientific Instruments uses 100 thermocouples to measure global solar radiation on a flat surface received from a complete hemisphere. Internal temperature variations are kept to a minimum and the sensitivity remains constant within ± 1% over at least a temperature range of -10°C to +40°C. In addition, two new solar integrators will accept inputs from one or two pyranometers. They integrate solar irradiance over time intervals of 10 min, 30 min, 1 h or 24 h and at the end of each preset period, a printout shows day number, real time, pyranometer calibration factor, period total and daily - or continuous total for each pyranometer.

Circle No. 108 on Reader Enquiry Card.

Particle sizer. The new Malvern Autosizer is an automatic, wide-range (10-3,000 nm) particle sizer which measures both size and diffusion coefficient. The instrument also incorporates automatic repeat measurement facilities for real-time studies of particle interactions such as agglomeration, or the effects of additives and pollutants.

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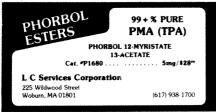


Epithelial cell culture. A new technique has been developed for culturing epithelial cells which avoids the use of y-irradiated fibroblasts as a biological substrate for the cells. The problem with the latter method is that the fibroblasts (feeder-cell) interfere biochemically with the epithelial cells. Now it is possible to culture epithelial cells without using such a substrate. The capsule of a bovine eye lens is fixed to a culture dish in such a way that epithelial cells can be cultured on it. This Epicult system is supplied by Sanbio.

Circle No. 110 on Reader Enquiry Card.

Data reduction. Five additional calculation procedures have been developed by LKB Instruments for the LKB-Wallac 1260 Multigamma counter data reduction system, based on the LKB spline program which constructs an accurate concentration curve using up to 10 standard points. Alternative data reduction methods may be selected, for example, curves of c.p.m. against concentration.

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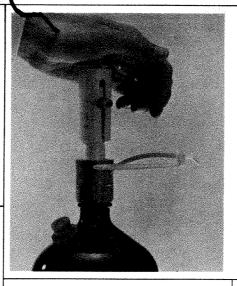
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Gas mixing. A range of gas mixing equipment from Air Liquide (UK) Ltd. makes possible the production of standard mixtures of accurately known composition. The range includes a dynamic mixer supplemented with a microcomputer that is already programmed with relevant data for all gases. The operator enters the required constituents and their concentrations, and the final filling pressure, the program then checks for flammability (rejecting flammable mixtures), calculates maximum filling pressure possible (in view of any considerable gases), performs all the other necessary calculations and then prints out the final mixture composition.

Circle No. 112 on Reader Enquiry Card.

IR spectrophotometer. The new model 983 IR spectrophotomer from Perkin-Elmer provides the basic performance needed to cover almost any IR application. It provides ratio recording, a choice of resolution from 10 to 0.5 cm<sup>-1</sup>, low noise levels and a range of 180 to 5,000 cm<sup>-1</sup>. Spectra are presented on a digital printer/plotter with axis annotation, with measurement conditions printout and peak listing as options. Built-in data handling includes spectral subtraction, accumulation, digital filtering and the ability to replot in different formats.

Circle No. 113 on Reader Enquiry Card.

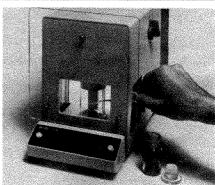
Digital display module. High-resolution vector graphics are possible using the HP 1345A digital display module from Hewlett-Packard. Intended for OEM applications with microprocessor-based instrumentation, typical applications for the module include spectrum and network analysers, waveform analysers, curve tracers, digital storage oscilloscopes and Fourier analysers. Integration of the HP 1345A into most instrument or system designs is simplified by the 16-bit binary interface which facilitates connection to 8-or 16-bit microprocessors. The advanced circuitry and electrostatic deflection CRT use < 25 W average power for increased reliability.

Circle No. 114 on Reader Enquiry Card.

Zippette dispensers. The range of Zippette dispensers from Jencons (Scientific) Ltd... has been extended by the addition of two new safety models, the 2.5-m1 and large capacity 100-m1 dispensers. Each model is simply adjusted to deliver a selected amount by turning a plastic adjuster knob against a non-fading, anti-scratch graduation. These dispensers can be used with acids and other aggressive liquids, as well as with organic solvents, and the entire dispenser can be autoclaved. The Zippette has no projecting glass valve assembly, no exposed glass syringe, and the inlet/outlet glass valve assembly is contained in a simple unit just below the screw neck in the reservoir bottle itself. This protects the operator in the event of a damaged valve. Circle No. 115 on Reader Enquiry Card.

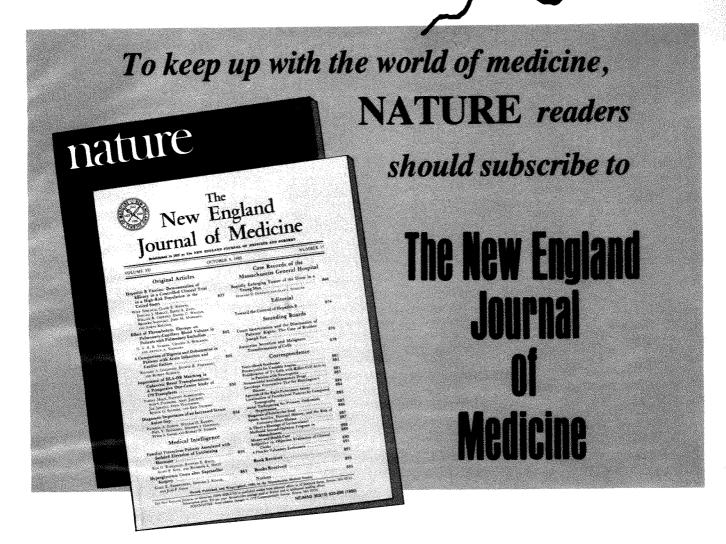
Fibre optic inspection system. A bulletin from Dolan-Jenner Industries describes a new fibre optic inspection system which provides virtually cold, high-intensity illumination and selective magnification from  $\times 2$  to  $\times 9$  for on-line inspection requirements. The model 370 features a lightweight illuminator source and a series of  $\times 2$ ,  $\times 3$  and  $\times 4$  magnifiers which can be used individually or stacked for desired magnifications. A fibre optic annular head transmits cool light for shadow-free illumination. The system is designed for inspection of microcomponents assembly detail, high-density solder connections, and low-magnification medical tissue examination. The compact illuminator housing will not exceed 80°F during continuous use and a 30-W quartz-halogen lamp provides a maximum of  $28.3 \times 10^3 \text{ W}$ cm-1 illumination.

Circle No. 116 on Reader Enquiry Card.



Electronic ultramicrobalance. The UM3 electronic ultramicrobalance from Mettler Instrument Corporation, intended for use in analytical laboratories, has a broad 1-15-mg electrical range, capacity of 3,005 mg and a readability of 0.1 µg. The balance features an easy-to-read digital display and built-in weight set specially protected against dirt and premature wear. Options include quick elimination of pan oscillations and automatic calibration. In addition, automatic transfer of weight values to a printer is possible using the Mettler 03 Data Output coupled with the UM3.

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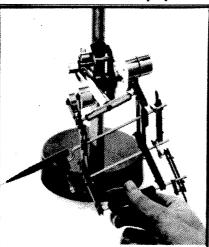
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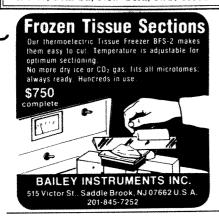
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Sample processing. The new universal computing sample-processing system from Hamilton, the Microlab 2000, is intended for liquid transfer in the clinical laboratory, including pipetting and diluting of samples. The Microlab 2000 is accurate even for samples as small as  $5 \mu l$  and reagent volumes up to 1 ml. The sample rack holds up to 500 tubes which may be thermostated and sealed, with up to 10



reagents in an adjacent rack containing four individually controlled magnetic stirrers. The unit also has a built-in wash station to minimize carry-over by washing the delivery tip both inside and out, and an integrated liquid detector will automatically set the depth of immersion of the pick-up tip to the minimum consistent with the volume to be aspirated. A disk-driven computer allows setting and control of all aspects of volume, positioning and sequence of operation; a print-out informs the user of operating conditions, working parameters and results.

Circle No. 119 on Reader Enquiry Card.

Capillary tubing. Precision 'thick septum theta' style borosilicate glass capillaries for the production of micropipette electrodes are available from W-P Instruments. This capillary tubing offers the advantages of better cell impalements due to a natural 'self-bevelling' spear-point tip, very low resistance when used as a single microelectrode and double microelectrodes with low trans-tip coupling. These capillaries are available with a standard 1.5 mm outside diameter.

Circle No. 120 on Reader Enquiry Card.

Photoelastic modulators. International announces the new PEM-80 series I and II photoelastic modulators. These feature a single control unit for multiple modulator heads covering the visible to UV range (series I) and the IR to far IR range (series II). Only low driving power is required (maximum 50 V) to obtain half-wavelength retardation, even in the far IR. The PEM-80 has an optical crystal/transducer assembly which resonates at different frequencies from 20 to 100 kHz depending on the modulator head. In turn, the periodic strain induced in the crystal causes a relative retardation between the two polarization components of a transmitted light beam. Photoelastic modulators are widely used in circular and linear dichroism measurements, spectropolarimetry, strain measurements and light modulation.

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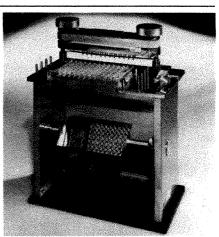
Monitoring of ATP release. A dual-sample lumi-aggregation instrument capable of simultaneously monitoring both platelet aggregation and ATP secretion on each of two samples is now available from Payton Associates. Platelet aggregation is measured photometrically while ATP release is determined by the firefly luciferin-luciferase reaction; curves are recorded on two dual-channel strip-chart recorders. Stirring speed and temperature are adjustable and continuously indicated. The 0.5-ml sample volume can be preheated in the incubation wells provided. Circle No. 122 on Reader Enquiry Card.

Capillary columns. The development of a new deactivation technique now enables WCOT glass capillary columns from Nanosep to be used at higher temperatures, considerably increasing the range of solutions which can be analysed. Operating temperatures > 300°C with minimum bleeding, and a more stable column makes possible the analysis of high-boiling point amines and phenols.

Circle No. 123 on Reader Enquiry Card.

Data logger. Columbus Instruments offer in kit form a 16-channel  $\pm 0.012\%$  accuracy (12 bits) professional data logger for scientific and industrial use. The kit model DL-1001 is based on Rockwell's AIM-65 computer expanded by 16-channel, 12-bit accuracy analog-to-digital converter, and has a calendar/clock for timing of printing intervals, etc. Voltage input ranges can be set to  $\pm 10V$ ,  $\pm 5V$  or 0 to  $\pm 10V$ . Conversion time of analog-to-digital converter is  $25\mu s$  per channel.

Circle No. 124 on Reader Enquiry Card.



Cell harvester. A compact, flexible apparatus for the rapid harvesting of cell cultures labelled with radioactive isotopes has been introduced by Uniscience Ltd. Its major advantages are the elimination of plastic tubing, economical use of filter paper, a special mirror for control of all harvesting procedures and the fact that one valve regulates flow of washing liquid. This apparatus harvests 24-h cultures simultaneously from a microtitration plate.

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(W543)A

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ASSISTANT/Associate Professor. Must have research interest in cellular and molecular aspects of hostparasite interaction. Postdoctorial experience in microbial physiology, molecular genetics, immunology, virology, or pathogenic organisms required. Teaching commitment in medical microbiology and graduate courses required. Closing date 15 February 1982. Send curriculum vitae, description of professional aspirations, and names of three referees to Dr Byron Burlingham, Department of Microbiology, School of Medicine, East Carolina University, Greenville, NC 27834. An Equal Opportunity through Affirmative Action ive Action (NW175)A Employer.

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Interested persons whould send applications, with résumé and names of three referees, to: Dr Helmut Ankel, Department of Blochemistry, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. An Equal Opportunity/ Affirmative Action Employer.

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(NW184)A

#### THE UNIVERSITY OF TEXAS AT ARLINGTON **CARBONATE PETROLOGIST**

The Department of Geology invites applications for a Tenure-Track or Tenured faculty position in carbonate petrology beginning fall 1982. Level of appointment and salary negotiable. A PhD and demonstrated research ability area required. Particular research interests could range from ancient/modern environmental analog studies to diagenetic/geo-chemical research. The successful applicant will be expected to teach graduate and undergraduate courses and supervise thesis research in his or her speciality, participate in the department's introductory- and general-geology teaching programs, and establish a vigorous research program leading to publications.

A résumé, 3 letters of recommendation and reprints of publications should be sent to Dr C I Smith, Chairman, Department of Geology, Box 19049, University of Texas, Arlington, Texas, 76019, by Feb 15, 1982. UTA is an equal-opportunity/affirmative-action employer. (NW186)A

#### **WELSH NATIONAL** SCHOOL OF MEDICINE

#### (UNIVERSITY OF WALES)

DEPARTMENT OF MEDICINE

#### **TECHNICIAN**

Applications are invited for the post of technician in the above Department at Heath Park, Cardiff. The post, which is initially for 2 years, involves laboratory work in the area of hypothalomic control of anterior pituitary gland function. Experience in protein chemistry for the separation, purification, identification and radiolabelling of peptides would be advantageous.

Salary on the scale for University Technical Staff Grade 4 — (£5,284 — £6,078 per annum); starting point for suitably qualified candidates could be up to £5,594 per annum.

Application form and further particulars (quoting Ref No M6/77) available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (Tel: Cardiff 755944 Ext 2296). Closing date for application 11th January, 1982. (089)A

CELLULAR PHYSIOLOGIST urgently needed. Teaching and research experience emphasized. For partipation in current work on cell culture of transformed cells. Please apply to the Chairman, Physiology Department, Ponce School of Medicine, P.O. Box 7004, Ponce, PR 00732 U.S.A. (NW150)A

#### DERBYSHIRE AREA **HEALTH AUTHORITY** CENTRAL DISTRICT

Derby City Hospital, Uttoxeter Road, Derby DE3 3NE

#### SCIENTIFIC OFFICER

Oncology Research Laboratory. Post doctoral Scientist required to join a small group undertaking research on the immunological aspects of Patients with malignant diseases. Applicants should have post-graduate experience in tumour immunology and cell cuture techniques and faimilarity with the techniques for production of mono-clonal antibodies for clinical use would be an advantage. The appointment is for 3-5 years in the first instance. Salary within the Scientific Officer (Whitley Council) scale.

Interested Applicants are advised to discuss this vacancy with Dr L C Shah, Tel (0332) 40131 ext 560.

Applications with full Curriculum Vitae and two Referees to Sector Administrator by 22nd January, 1982. Ref No 561. (103)A

#### **COLLEGE OF ENGINEERING AND** APPLIED SCIENCE

The University of Wisconsin-Milwaukee invites applications and nominations for the position of Dean of the College of Engineering and Applied Science. The University, with 27,000 students, is one of two doctoral level campuses in the University of Wisconsin System with a strong emphasis on graduate education and research. UWM is located in the heart of the state's largest metropolitan area and the center of its manufacturing and business enterprise.

The College of Engineering and Applied Science has five departments: Civil Engineering, Electrical Engineering and Computer Science, Industrial and Systems Engineering, Materials Engineering, and Mechanical Engineering, offering programs leading to bachelor through doctoral degrees.

The Dean is responsible for administrative leadership of the College. Duties include the continuing development of ongoing research programs, liaison with the industrial community, and promotion of continuing professional education programs.

Candidates should have a doctoral degree in engineering or in an applied science or demonstrated equivalent, and qualifications for a tenured faculty appointment in one of the departments of the College. The position will be available by the Summer of 1982.

All nominations and applications for the position (including résumé and the names of at least three references) should be postmarked by March 1, 1982, to: Dr Robert C Eidt, Chairperson, Search and Screen Committee, The University of Wisconsin-Milwaukee, Department of Geography, Milwaukee WI 53201.

An Equal Opportunity/Affirmative Action Employer. (NW159)A

#### **UNIVERSITY OF** SOUTH FLORIDA

DEPARTMENT OF BIOLOGY The Department anticipates TWO TENURE-EARNING **FACULTY POSITIONS** 

at the assistant professor level commencing August, 1982. These are: 1) an animal virologist; 2) a comparative physiologist with an interest in the lower vertebrates. A PhD is required and post-doctoral experience is preferred.

Applicants should submit a curriculum vitae and statement of research interests and should solicit three letters of recommendation to be sent to: Chairman, Search Committee, Department of Biology, University of South Florida, Tampa 33620. Deadline for submission is 15 February, 1982. The University of South Florida is an affirmative action, equal opportunity employer, and applications from women and minority individuals are encouraged.
(NW178)A

#### UNIVERSITY OF PAPUA NEW GUINEA

Applications are invited for the post of

## PROFESSOR OF CHEMISTRY

The appointee will be the Academic Head of Department, and will be eligible to be elected as Chairman (Administrative Head). As such, applicants should have wide capabilities and extensive experience in both teaching and research. The new Professor will be expected to contribute significantly to the research of the Department and should have wide research interests. Present research in the Department concentrates on the applications of Chemistry to development in Papua New Guinea.

Fields of interest include: chemical education; coordination chemistry; environmental and analytical chemistry; food chemistry; marine chemistry; natural products; phytochemistry of medicinal plants; and structural chemistry. The research interests of the Professor need not be confined to these areas, but he/she should be willing to apply his/her research to the needs of the country. A major responsibility of the Department is in undergraduate teaching. This includes relatively elementary service teaching, but also more advanced courses for Chemistry and other Science Students.

Applicants should also have a strong interest in the problems of teaching chemistry in Papua New Guinea at all levels covered in the University. The Department is committed to the training of National Academics and a significant contribution will be expected in this area of advanced training. Previous experience of teaching and research in a developing country in a tropical environment will be considered an advantage.

Salary: K 21,295 pa (£1 sterling = K 1.27). Three-year contract; gratuity; support for approved research; rent-free accommodation; family passages; baggage allowance; leave fares after 18 months service; education subsidies; salary continuation scheme to cover extended illness or disability.

Applicants who wish to arrange secondment from their home institutions will be welcomed. Detailed applications (2 copies), including a curriculum vitae, a recent small photograph and naming 3 referees, should be sent to the Assistant Secretary (Staffing), University of Papua New Guinea, Box 4820, University PO, Papua New Guinea to arrive no later than 31 January 1982.

Applicants resident in UK should also send 1 copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 10 Spring Gardens, London SW1A 2BN. Further details are available from either address. (093)A

## UNIVERSITY OF ARIZONA

MULTIPLE MIRROR TELESCOPE OBSERVATORY

# MMT INSTRUMENT SPECIALIST

We invite applications for a vacant position of Instrument Specialist at the Multiple Mirror Telescope in Arizona. The MMT is a telescope of highly novel design largely devoted to state of the art optical and infrared astronomical observations. The instrumentation is among the most advanced and relies strongly on modern digital technology. The instrument specilist will be responsible for the installation, checkout, and operation of the facility instruments at the MMT. At the moment these consist of two spectrographs and an infrared photometer. In the future other instruments, specifically a CCD camera, will be added. The person filling this position will have as primary responsibility the care of the IR photometer. A background in infrared astronomy is therefore very desirable. Minimum academic requirement is a Bachelors degree in physics or related fields. The position requires working at the telescope location at an altitude of 8,600 feet at a two hour drive from Tucson.

Applications should be sent to Dr J M Beckers, Multiple Mirror Telescope Observatory, University of Arizona, Tucson AZ 85721 (tel. 602-626-1558) before February 15, 1982. The MMTO is a joint venture of the University of Arizona and the Smithsonian Institution. The University of Arizona is an Equal Employment Opportunity/Affirmative Action Employer. (NW179)A

# ROSWELL PARK MEMORIAL INSTITUTE MOLECULAR IMMUNOLOGY

a comprehensive cancer research center has available three-tenure track positions which are open in the Department of Molecular Immunology. The department is expanding into the areas of idiotype manipulation and recombinant DNA technology. One of the positions is at the senior, (full professor equivalent) level and two are at the junior level. No formal teaching is required at the Institute, but graduate students are available through the institutional graduate program. The candidate for the senior staff position will be consulted in the selection of the junior candidates.

Applications should be forwarded to Heinz Kohler, Chairman of the Department of Molecular Immunology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14263. An Affirmative Action/Equal Opportunity Employer. (NW170)A



#### JANSSEN PHARMACEUTICAL LIMITED

# SENIOR CLINICAL RESEARCH ASSOCIATE

To enable us to continue to expand our clinical research programme on our range of anti-fungal products, we require a science graduate, preferably with post-graduate research experience and at least two years experience working within the pharmaceutical industry industry.

The successful applicant will act as Senior Clinical Research Associate and will initiate, supervise and progress a clinical trial programme required for new developments in our anti-fungal range. The position reports directly to the Director of Clinical Research.

Energy, judgement, personality and good communication skills will be rewarded by an excellent salary (according to experience), a company car and good working conditions in the pleasant location of Marlow.

If this opportunity is of interest to you, and you would like to know more about us, please telephone or write with full Curriculum Vitae to:

Mr. M. B. Emanuel,
Director of Clinical Research,
Janssen Pharmaceutical Limited,
Janssen House,
Chapel Street,
MARLOW, Bucks.
SL7 1ET.
Tel: Marlow 71744

Last date for applications Friday 15 January, 1982.

(091)A

#### YARMOUK UNIVERSITY Irbid, Jordan

## TEACHING POSITIONS

Yarmouk University invites applications for teaching posts in the fields of specialization listed below for the academic year 1982/1983:

— Biology: (Comparative Anatomy, Molecular Biology, Plant Physiology, Virology).

- Physics: (Solid State Physics (Experimental), Quantum Electronics, Astronomy (Observational)).

Candidates should hold a PhD. Annual Salary ranges from \$16,000 to \$28,000 for nine months service. Jordanian income tax nominal. Fringe benefits include medical and life insurance, furnished housing for reasonable rent, and travel air tickets for appointee and family (spouse and children under eighteen). Extra remuneration is paid for summer teaching. A model school is available with nursery section and two programs of instruction, one for Arabic speaking children and the other for English speaking children.

Complete dossier (transcript, three letters of reference, copies of degrees) to be sent not later than end of January 1982, to: The Dean, Faculty of Science, Yarmouk University, Irbid, Jordan. (W541)A

# UNIVERSITY OF THE WEST INDIES Trinidad

#### Applications are invited for the post of LECTURER/ASSISTANT LECTURER IN BOTANY

in the Department of Biological Sciences. Applicants should hold a good Honours Degree in Botany and preferably have a higher degree.

Duties of appointee will include teaching undergraduate Botany courses including Plant Morphology and Anatomy and Economic Botany.

Salary scales (to be reviewed): Lecturer TT\$ 29,784 — 43,752 pa. Assistant Lecturer TT\$ 24,156 — 26,484 pa. (£l sterling = TT\$ 4,69). FSSU, Unfurnished accommodation or housing allowance. Family passages. Study and Travel Grant.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent as soon as possible to the Secretary, University of the West Indies, St Augustine, Trinidad.

Applicants resident in UK should also send I copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 10 Spring Gardens, London SWIA 2BN.

Further details are available from either address. (096)A



#### **MOLECULAR BIOLOGY**

The Microbiology Department at Case Western Reserve University invites applications for several tenure-track faculty positions. Although we encourage applications from candidates with strong credentials in any area of Molecular Biology, we are especially interested in applicants who propose to study regulation of gene expression, or molecular biological aspects of virology of immunology. Experience in recombinant DNA techniques is desirable. These appointments provide excellent opportunities to develop independent research and teaching programs in an expanding medical school department. Send curriculum vitae, a statement of research and teaching interests and the names of four references to Dr. Fritz Rottman, Chairman, Department of Microbiology, Case Western Reserve University, Cleveland, Ohio 44106.

An Equal Opportunity/Affirmative Action Employer
(NW188)A

#### UNIVERSITY OF GLASGOW DEPARTMENT OF ONCOLOGY

Applications are invited for the post of RESEARCH ASSISTANT

in the Department of Oncology. The post is suitable for graduates in Biochemistry, Cell Biology or related fields. The project, supported by the Cancer Research Campaign for a period of three years, involves studies on mechanisms of tumour cell resistance to cytotoxic drugs using animal models and cultured tumour cells.

The initial salary is £5,289 per annum, plus U.S.S. benefits.

Applications with curriculum vitae and names of two professional referees should be sent to Dr S Kaye, Department of Oncology, University of Glasgow, 1, Horslethill Road, Glasgow Gl2 9LY, to arrive by 11th January, 1982. (098)A

PHYSIOLOGY Faculty Position: Assistant Professor tenure-tract position for July, 1982. An interest and post-doctoral research experience in gastrointestinal physiology, particularly in peptides of the gastrointestinal tract and their regulatory role, is preferred. Candidates will be expected to teach physiology of the digestive tract to medical students and to participate in graduate courses and to develop an independent research program. Applicants should submit curriculum vitae, reprints and statement of research interests, and teaching experience, and have three letters of recommendation sent to: Dr Robert E Forster, Chairman, Department of Physiology, School of Medicine, University of Pennyslvania, Philadelphia, Pa 19104. An equal opportunity/affirmative action employer. (NW164)A

## ICHTHYOLOGY JOB DESCRIPTION

Cornell University solicits applications for a tenure-track position, preferably an Assistant Professor, in the Section of Ecology & Systematics to begin September 1982. We seek a Systematic Ichthyologist with broad intergets in vertebrate biology and a strong research program. The successful candidate will participate in curation of the Ichthyology Collection. Teaching responsibilities include an upper-level course, and participation in the graduate program in vertebrate biology.

Applications must include a curriculum vitae, names of three references, and will be reviewed February 1, 1982. Send application to: Dr W N McFarland, Section of Ecology and Systematics, Cornell University, Ithaca, New York 14850. Cornell University is an affirmative action/equal opportunity employer.

(NW157)A

#### UNIVERSITY COLLEGE LONDON School of Medicine

#### **TECHNICIAN**

required to work with Dr R A Knight and Dr M L Snaith on immunological aspects of systems lupus erythematosus in man, in the Rayne Institute of University College Hospital.

Applicants should have experience of tissue culture, and in vitro tests of human lymphocyte function. Appointments is for two years, and the starting salary is on the third year of the MLSO scale.

Applications with a curriculum vitae and names of 2 referees should be sent to Mr Blackman, Personnel Officer, Technical Staff, University College, London WC1. (095)A

#### **MICROBIOLOGY**

Applications are invited from individuals with demonstrated excellence in microbial biochemistry or molecular biology for a tenure-track assistant professorship in the Department of Microbiology at the Medical College of Wisconsin. Applicants must have postdoctoral research training and be able to develop an independent research program, teach medical students, and instruct garduate students.

Send résumé, summary of proposed research, and names of references to: Search Committee, Department of Microbiology, Medical College of Wisconsin, PO Box 26509, Milwaukee, Wisconsin 53226. Affirmative Action/Equal Opportunity Employer.

(NW176)A

#### ANIMAL BIOCHEMIST

The Department of Agricultural Biochemistry of the University of Nebraska-Lincoln. Tenure-track assistant or associate professor. Postdoctoral experience and research interests in any area of basic biochemistry related to the growth and reproduction of domestic animals is desirable. This 12-month position, primarily in the Experimental Station, includes teaching duties. Résumé and names of three references should be sent by February 28, 1982 to: J.M. Daly, 206 Agricultural Biochemistry Hall, The University of Nebraska, Lincoln, NE 68583-0718. Phone (402) 472-2932. An Equal Opportunity/Affirmative Action Employer. (NW187)A

#### UNIVERSITY COLLEGE LONDON School of Medicine

#### 1st year POSTDOCTORAL SCIENTIST

required to work in a laboratory headed by Dr R A Knight and Dr M L Snaith on immunological dysfunction in human systemic lupus erythematosus.

The laboratory is in the Rayne Institute of University College Hospital, London.

Its main interests are in pathways of suppressor T cell generation, the role of interferons in these pathways, and on the nature of the suppressor deficit in lupus patients.

Applications should include a curriculum vitae, and the names of 2 referees, and should be sent to Dr R A Knight, Dept of Rheumatology, University College Hospital, London WC1. (094)A

#### TOXICS SCIENTIST

Ph.D. to direct technical aspects of Toxic Chemicals Program with national environmental organization in Washington, D.C. Salary competitive.

Apply in writing to: Environmental Defense Fund, 444 Park Avenue South, 9th Floor, New York, N.Y. 10016.

(NW181)A

POSTDOCTORAL position available beginning June 1, 1981 in the Developmental Biology Center, University of California, Irvine. Recent PhD required preferably in the areas of cell biology, molecular biology, developmental biology, or genetics. Research will be conducted in the laboratories of P J Bryant, J L Marsh (molecular genetics of *Drosophila* development, using molecular cloning and/or hybridomas), or R Cambell (cell behaviour and morphogenesis). The University of California is an equal opportunity/ affirmative action employer. Submit resume and brief description of research interests and experience, together with the names of three individuals willing to write on your behalf, to: Peter J Bryant, Developmental Biology Center, University of California 92717 by March 1, 1982. (NW185)A

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Jean Neville
Jean Neville
4 Little Essex Street
London WC2R 3LF
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE Cathy Moore 15 East 26 Street 15 East 70010 New York, NY 10010 New York, NY 10010 Tel: (212) 689 5900 Tel: (212) 68 497 Telex: 66 8 497

TORONTO OFFICE

Peter Drake Associates
Peter Drake Associates
32 Front Street West
32 Front Ontario
200 Toronto, Ontario
M5J 1C5
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Tel: (416) 364 1623

#### SHEFFIELD AREA **HEALTH AUTHORITY** (TEACHING)

**Royal Hallamshire** Hospital WHOLE TIME **GRADUATE NON** MEDICAL SCIENTIFIC **OFFICER** 

BSc Honours Graduate with a 2:1 Biological Science degree is required to investigate Haemostatic Mechanisms with particular emphasis on Platelet Biochemistry in patients with Renal Disease in the Department of Renal Medicine and Haematology at the Royal Hallamshire Hospital, Sheffield. This research post is funded for one year in the first instance, with possible extension to three years, at a starting salary of £5,667 per annum, depending upon experience and degree.

Applications stating age, qualifications, present and previous appointments (with dates) giving names and addresses of two referees to the Area Personnel Officer, Westbrook House, Sharrow Vale Road, Sheffield S11 8EU by 15th January 1982. (102)A

#### THE INSTITUTE FOR **ADVANCED STUDY**

will have several openings for members in theoretical physics and astrophysics for the academic year 982-83. The positions are at a postoctoral or higher level and appli-ints will be selected on the basis of neir ability to do research in the areas elementary particles, matheatical physics, astro-physics sma physics, general relativity and itistical mechanics. Preference is ven to candidates who have ceived their PhD within the last ear or two.

Postdoctoral members frequently collaborate with each other, with faculty members at the Institute or Princeton University, and with esearchers at other institutions

Appointments are usually for no more than two years and support is typically full salary for postdoctorals and half salary for more senior persons. Women and minorities are acouraged to apply

For more information write to Ms Valerie Nowak, Administrative Officer, School of Natural Science, he Institute for Advanced Study, Princeton, New Jersey 08540. Applications should be received by December 15, 1981. (NW1006)A

# Training Fellowships 1982-83

The Medical Research Council's Training Fellowship scheme enables **medical and dental graduates** at any stage in their career up to and including Senior Registrar, Lecturer or equivalent levels, and science graduates with post graduate or postdoctoral experience to gain specialised research training in the biomedical field in the UK

Applications are now invited from suitably qualified candidates for awards to be taken up in the 1982-1983 academic year. Fellowships may be held for periods of from six months to three years (subject to annual review).

ELIGIBILITY REQUIREMENTS: candidates should have been ordinarily resident in the UK throughout the period of 3 years immediately preceding the application date. Graduates in medicine and dentistry: from post-registration up to Senior Registrar or equivalent academic levels. Graduates in science: PhD/DPhil (or in certain circumstances MSc plus at least 3 additional years'postgraduate experience).

TRAINING PROGRAMMES: proposals may be submitted for specialised research training or in the case of medical/dental graduates for training in a basic subject relevant to a particular clinical interest. Fellowships are also available to provide support for medical/dental graduates to attend one-year researchorientated courses leading to an MSc.

EMOLUMENTS: appropriate point, with increments, on the current NHS medical, equivalent dental or current university non-clinical lecturer scales.



Application forms are available on request from the Medical Research Council, Training Awards Group, 20 Park Crescent, London W1N 4AL (tel. 01-636 5422 ext. 448). Closing Date for receipt of applications: 28 February 1982. Late applications will Medical Research Council not be accepted.

# **MRC Celltech Fund** Research Fellowship

Kingdom is offered from October 1982. Applicants should have proven research ability in a subject in the MRC field and be below the age of 32 on 30 September 1982; there is no restriction as to nationality or domicile. Those interested should discuss their proposal with the Director of the MRC laboratory in which they wish to work.

The MRC Celltech Fund, which is administered by a subcommittee of the Council, derives its revenue from royalties payable by Celltech Ltd on sales and services based on research in MRC establishments



Further details and application forms are available from Mr. B. C. Dodd, Medical Research Council, 20 Park Crescent, London W1N 4AL. Closing date for receipt of of applications: 28th February, 1982. Late applications will not be accepted.

(085)E

fellowships continued on page xviii

Please mention

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when replying to these advertisements

#### WORKSHOP

**HUMAN TUMOUR 'STEM' CELL CLONING -**2ND WORKSHOP

**IMPERIAL CANCER RESEARCH FUND** Lincoln's Inn Fields, London, WC2 March 25-26, 1982

This workshop will deal with (1) Practical (2) In Vitro drug testing and (3) Clinical aspects of these techniques. Dr S Salmon (Tucson, USA) and Dr K M Tveit (Oslo, Norway) have been invited. Participation will be limited to clinicians and scientists with practical and/or clinical experience of drug sensitivity testing.

Those wishing to contribute are invited to send details to Bridget T Hill at the above address by January 25th, 1982. For more information 'phone John Masters, 01-836 9611. (088)V

#### CONFERENCES and COURSES

#### **FEBS ADVANCED COURSES**

No.82/01. "Biomolecular Electron Microscopy (BIOMOLEM 1982)" Ulm, FRG, 25 March-3 April 1982

The course, organized by A.K. Kleinschmidt and G. Klotz, will be conducted at an advanced level covering selected methods of biomolecular electron microscopy. It will include specialized lectures, with short communications by participants, and lectures on general topics. Laboratory practical sessions will be on topics of the participant's choice, and will include electron microscopy of nucleic acids, protein and nucleoproteins, membranes and lipoproteins, and molecular studies in biological supraorganization. Course fee, including accommodation and meals: DM 600. Further info and application forms (to be returned by 1 February 1982): Dr G. Klotz, Microbiology Department, University of Ulm, D-7900 Ulm, Postfach 4066, FRG.

No.82/02. "Biochemistry and Genetics of Yeasts" Madrid, Spain, 5-24 July 1982

A course of lectures, seminars and practical work on the potentialities of yeast as a model eukaryotic system for studying enzyme regulation and gene expression. Topics: energetics, physiological inactivation of enzymes, compartmentalization and regulation, complementation and recombination, yeast transformation, and cloning of yeast genes Course fee, including accommodation and breakfast: about 35,000 ptas. Further info and applications (before 28 February 1982): Dr C Gancedo, Instituto de Enzimología del C.S.I.C., Facultad de Medicina de la Universidad Autónoma, Arzobispo Morcillo, 4, Madrid-34, Spain.

# No.82/07. "Replication of Bacterial Plasmids" Odense, Denmark, 2-18 August 1982

A laboratory course for 12 students, organized by K Nordström and S Molen, concentrating on plasmid replication and its control, plasmid partitioning at cell division and the correlation between these processes and plasmid incompatibility. The course will include a theoretical part organized as mini-symposia with short communications by participants, and a practical part introducing various techniques and experimental approaches used in plasmid research as well as in work where plasmids are used as experimental tools. The main biological material used will be *E. coli* and the plasmids R1 and ColE1. Registration fee, including accommodation: DM400. Further info and application forms (to be returned by 31 March 1982): Dr Kurt Nordström, Dept. of Molecular Biology, Odense University, Campusvej 56, DK-5230 Odense M, Denmark.

#### No.82/05. "Structure and Function of Plant Genomes' Erice, Sicily, Italy, 1-11 September 1982

course of lectures, discussions and poster presentations, for about 120 students. Main topics: Structure, organization and expression of the nuclear genome, the chloroplast genome, and the mitochondrial genome in higher plants; Biosynthesis of plant storage proteins; Genetic manipulation of higher plants; Nitrogen fixation; Molecular biology of host-pathogen interaction; Molecular biology of plant viruses. Living expenses: US\$450 approx. All participants will be expected to present a poster. Applications, with a curriculum vitae, a provisional poster abstract, and other information to aid evaluation, should be sent in duplicate to: Dr Orsola Tiboni, PO Box 202, 27100 Pavia, Italy, to arrive not later than 15 March 1982

#### No.82/04. "Structure and Metabolism of Glycoconjugates" Lille, France, 6-18 September 1982

The fee for this course, including accommodation and meals, will be FF 2000 approx. Applications should be sent by 1 April 1982 to: Professor J Montreuil, Université des Sciences et Techniques de Lille I, Laboratoire de Chimie Biologique, 59655 Villeneuve d'Ascq Cédex, France, from whom further information may be obtained. (092)C

Please mention

# nature

when replying to these advertisements

#### **INSTITUTES OF** PSYCHIATRY, NEUROLOGY AND OPTHALMOLOGY

(British Postgraduate Medical Federation, University of London)

#### MSc IN **NEUROCHEMISTRY**

This is a ONE-YEAR Course leading the degree of MSc in Neurochemistry. It offers graduates in science and medicine training in basic and applied neurochemistry. Course consists of lectures, seminars and laboratory work and, in the second and third terms, research on a neurochemical topic.

Applicants should have a First or Second Class Honours degree in Biochemistry or a cognate subject, or be medically qualified. The Course is recognsed by the Medical Research Council as an Advanced Course for the award of Training Scholarships to suitably qualified applicants.

Applications for the Academic Year October 1982 — July 1983 should be addressed to the Secretary, Course in Neurochemistry, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, England. (097)C

#### **TENDERS**

#### **ROYAL BOROUGH OF** KINGSTON UPON THAMES **Directorate of Education** and Recreation

#### Kingston Polytechnic

Tenders are invited for the supply of multinuclear Fourier Nuclear Magnetic Resonance Spectrometer at the Polytechnic. The general specification includes:

a) An ability to measure proton (hydrogen 1) spectra\_at a frequency between 80 MHZ and 100 MHZ;

b) Multinuclear observation capability including the ability to obtain spectra for Carbon — 13, phosphorus — 31 and nitrogen —

c) Dedicated computer, minimum capacity 16K, and software package for spectrometer control and data processing;

d) Foreground/background capability or the equivalent, for simultaneous spectrum acquisition and data processing;

e) Variable temperature operation 150°C to + and the range -150°C.

Potential tenderers should apply for tender documents and a more detailed specification to the Administration Manager, Directorate of Education and Recreation, Guildhall, Kingston upon Thames not later than 31 December, 1981. (100)T

FELLOWSHIPS continued from page xvii

#### **DEPARTMENT OF EDUCATION, DUBLIN** POST-DOCTORAL RESEARCH FELLOWSHIPS 1982/83.

Applications are invited for research fellowships in science (excluding medical and social science) and engineering tenable in any one of the following institutions in Ireland:-

University College, Dublin.

University College, Cork. University College, Galway. Trinity College, Dublin. St. Patrick's College, Maynooth.

The National Institute for Higher Education, Dublin. The National Institute for Higher Education, Limerick.

An Foras Talúntais

(The Agricultural Institute), Dublin 4.

The Institute for Industrial Research and Standards, Dublin 9.

Fellowships will be tenable for a period of two years with the possibility of an extension for a third year.

Applicants should hold the degree of PhD or have equivalent research experience. Applications will be considered from engineers or technologists with suitable industrial experience. Candidates must be under the age of 30 years and preferably under the age of 28 years on 20 March, 1982.

Appointments will be effective from a date not earlier than 1st October, 1982. The initial salary, which will depend upon qualifications and experience, will normally be within the range £5,392 to £6,081 per annum, but awards of up to £7,056 may be made in the case of engineers and technologists whose industrial experience or other special circumstances warrant it.

Full particulars of the scheme including application forms may be obtained from The Secretary, Department of Education (Headquarters Section 3), Marlboro' Street, Dublin 1, to whom applications for Fellowships should be forwarded. The latest date for the receipt of applications is 20th March, 1982. (W542)E

## DICTIONARY OF THE HISTORY OF SCIENCE

Edited by W.F. Byrnum E.J. Browne Roy Porter

# Is soon of the state of the sta

Is there a gap in your bookcase? Until now, there hasn't been a dictionary devoted exclusively to the history of scientific ideas. And however you are involved in today's scientific world, you will agree that your present work and scientific attitudes are inevitably influenced by and built on those from the past.

Science and its key doctrines – from quantum theory to genetics – are central to the modern world; yet daily it changes and develops, becomes more technical and diverse. What is needed is a reference book that enables you not only to master the past but also to keep you in touch with the theories underlying the most recent scientific and technological developments.

The Dictionary of the History of Science fills the gap.

#### Authoritative

It is the work of three editors: W.F. Bynum, E.J. Browne and Roy Porter; of ten subject editors – including Michael Hoskin, Richard Burkhardt, William Brock, Steven Shapin, Roger Smith, Eric Aiton, Roy Bhaskar, John Heilbron – and over 90 contributors, all authorities in their field. It explains the core features of Western science and their development. 700 entries, alphabetically arranged, cover such fields as astronomy, biology, chemistry and earth sciences, the historiography, philosophy and sociology of science; human sciences, mathematics, medicine and physics.

Substantial concepts such as Evolution and Light are given generous entries, while more specialized subareas like Neo-Darwinism and Light Velocity are allocated smaller amounts of space. All are cross-referenced back and forth.

#### Comprehensive

Although the Dictionary is principally concerned with the Western scientific tradition of the last 500 years, it touches, too, on the central scientific ideas of classical and medieval times, and of other cultures. In addition, it features such topics as the development of technology and clinical medicine, and of important scientific instruments like the astrolabe and the thermometer.

Our understanding of the processes of scientific discovery and change is often controversial. But however you view them, it is important that you have easy access to all the ideas you are building on today, in the most scholarly, up-to-date and comprehensive detail.

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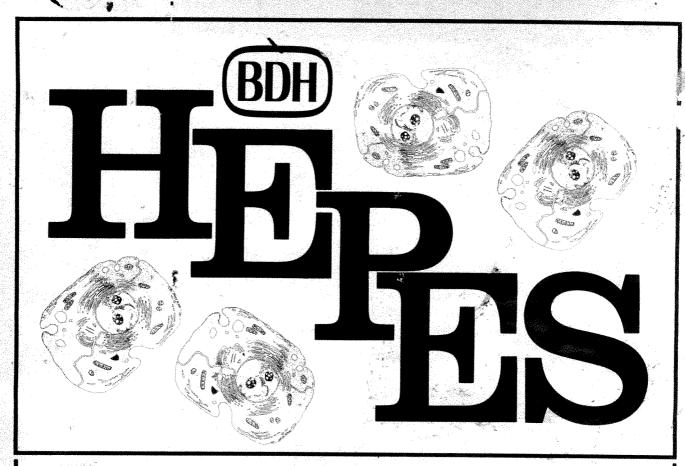
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